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TAMPERE UNIVERSITY OF TECHNOLOGY

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*IN VITRO* AND MECHANICAL PROPERTIES OF BIOACTIVE-  
GLASS/POLYMER COMPOSITES  
Master of Science thesis

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## ABSTRACT

**SRIJANA GHIMIRE:** *In vitro* and Mechanical Properties of Bioactive- Glass/polymer Composites

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Polymer/bioactive glass composites are getting more interest nowadays in bone tissue engineering and orthopedic applications where polymers are used as matrix material and bioactive glasses are used as either filler material or as coating. The idea of combining bioactive glasses with biodegradable polymers is to develop composites that takes an advantages of osteoconductive properties of bioactive glasses and their strengthening effect on polymer matrices. Such composites are expected to have superior mechanical properties than the neat unreinforced polymer and to improve structural integrity and flexibility over brittle glasses for eventual load-bearing applications.

The thesis is based on composites consisting of PLA 70/30 as matrix polymer and bioactive glass as a filler material prepared by twin-screw extrusion method. The polymer matrix was combined with different weight percent of silicate and phosphate based bioactive glasses. Fourier Transform Infrared Spectroscopy (FTIR) was used to analyze the surface and structural changes in the composites upon immersion in the buffer solution. Mass change, water uptake measurement and FTIR indicates that with increasing the filling content the water absorption was increased. The changes were more drastic with the phosphate based composites compared to the silicate one. Ca release profile was analyzed by *Atomic Absorption Spectroscopy (AAS)* and was found to increase with immersion time.

The mechanical properties of all investigated glasses were found to first decrease, as expected upon degradation of the composite. However, the bending strength increased after two weeks of immersion was unexpected, especially since the shear strength was not found to greatly change over the course of the test, and calls for more in-depth analysis. Finally, molecular weight of the polymer was found to decrease upon immersion up to two weeks and remained stable for longer immersion time in silicate based composite while in case of phosphate based composites, molecular weight was found to increase drastically after two weeks of immersion, raising the question of the impact of P release on the cross linking of the polymer chains. Overall a 30 weight % of glass within the composite was found to be optimum to maintain high mechanical properties, ductility, while providing significant Ca release, promising indication of the bioactivity.

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## LIST OF SYMBOLS AND ABBREVIATIONS

AAS	Atomic absorption spectroscopy
BG	Bioactive Glass
FTIR	Fourier transform infrared spectroscopy
g/h	Gram Per Hour
GPC	Gel Permeation Chromatography
HA	Hydroxyapatite
h	Hour
Mn	Number Average Molecular Weight
Mw	Weight average molecular weight
MWD	Molecular weight distribution
PLA	Polylactic acid
SR	Self-reinforced
TGA	Thermogravimetric Analysis
T <sub>g</sub>	Glass transition temperature
T <sub>m</sub>	Melting Temperature
WA	Water Absorption
WL	Weight loss
wk	Week
Wt. %	Weight percentage



# 1. INTRODUCTION

Biodegradable polymers can be defined as materials whose physical and chemical properties undergo deterioration and complete degradation when exposed to biological environment. The degradation products of such polymer are excreted through the normal metabolic activity of living system. Biodegradable polymers has been widely used in medical application such as degradable suture, controlled drug delivery, coating on metallic implant etc. (Babu *et al.* 2013) Among many biodegradable polymers, Poly lactic Acid (PLAs) are commercially available interesting thermoplastic polymers which has many unique characteristics such as good transparency, glossy appearance, high rigidity and ability to tolerate various types of processing conditions. PLAs belongs to the family of aliphatic polyesters with the basic constitutional unit being lactic acid. The lactic acid monomer, the hydroxyl carboxylic acid, can be obtained through bacterial fermentation from corn (starch) or sugars that are obtained from renewable resources. PLA can be synthesized from lactic acid by direct polycondensation reaction or ring-opening polymerization of lactide monomer (Babu *et al.* 2013). Despite its unique properties, it lacks the osteoconductive and bioactive feature which limits its application in orthopedic application.

Bioceramics are a class of ceramics which are used for repair and replacement of diseased and damaged parts of musculoskeletal systems. Bioceramics have been used for medical applications, mainly for implants in orthopedics, maxillofacial surgery and for dental implants. Some bioceramics, like bioactive glass and  $\beta$ - tricalcium phosphate ( $\beta$ -TCP), have capability of forming direct chemical bonds with bone or even with soft tissue of a living organism. (Thamaraiselvi & Rajeswari *et al.* 2004). However, the brittleness and low fracture toughness of bioactive glass limits its application in load bearing application in the biomedical field.

Although, fabrication of polymers into complex shapes and porous structures is easy, they lack a bioactive function and are too flexible and weak to meet the mechanical demands in bone regeneration which limits their application in orthopedic application while on other side, the brittle nature of bioactive glass has limited the application in loadbearing application regardless of its bioactive nature. So, to overcome the limitations of both polymer and bioactive glass, the idea of bioactive glass and polymer composite has been developed. By combination of bioactive bioceramic and biodegradable polymer, osteoconductive and bioresorbable composite with tailored mechanical properties and ease of shaping can be developed.

The thesis is based on composites which were prepared by combining PLA 70/30 with different weight percent (wt. %) of silicate (13-93) and phosphate (Strontium 50) based bioactive glass. The rod-shaped composite was prepared by twin screw extrusion method. TRIS buffer was used as dissolution solution for *in vitro* test series. The aim of this thesis was to compare the mechanical and hydrolytic behavior of different composite samples at different immersion time point. *In vitro* properties of sample were analyzed in terms of change in pH, mass loss, water absorption and mechanical testing (3-point bending and shear test). Composites were also characterized using Fourier Transform Infrared Spectroscopy (FTIR), Atomic Absorption Spectroscopy (AAS), Gel Permeation Chromatography (GPC) and Thermogravimetric Analysis (TGA). Characterization of different composite should help in defining the most suited bioactive glass type and filler content to tailor properties based on the desired applications.

In this thesis, an in-depth literature review has been conducted and is presented in Chapter 2. In the literature review, bioactive glass, biodegradable polymer, polymer/glass composites and advantages and disadvantages of polymer and glass have been discussed. Special attention has been brought to the state of the art on polymer/glass composite. Impact of glass compositions and filler content on various properties of the composites are discussed. Chapter 3 reports the materials and method. In this section the glass and polymer types studied along with the preparation methods and characterization techniques employed are described. The main results obtained over the course of the thesis are reported and discussed in Chapter 4. The main conclusions are reported in Chapter 5.

## 2. LITERATURE REVIEW

### 2.1 Bioceramics

Bioceramics are groups of ceramics that are commonly used for the repair and reconstruction of diseased or damaged parts of the musculoskeletal system. Bioceramics may be bioinert such as alumina and zirconia. They can be resorbable like tricalcium phosphate, or bioactive like hydroxyapatite, bioactive glasses, and some glass-ceramics. Bioceramics are used as bulk materials as in the case of hip implant or as porous structure (scaffolds or coatings) when tissue ingrowth and firm bond with tissue are required. Bioceramics have been used in replacements for hips, knees, teeth, tendons, and ligaments and repair for periodontal disease, maxillofacial reconstruction, augmentation and stabilization of the jaw bone, spinal fusion, and bone repair after tumor surgery. (Hench, 1998) We will focus more on resorbable and bioactive bioceramics in the following section.

#### 2.1.1 Bioactive glass

Bioactive glasses were introduced by Hench in the early 1970s. A bioactive material has been defined as a material that has been designed to induce specific biological activity (Williams, 2009). Bioactive material undergoes specific surface reactions, when implanted into the body, leading to the formation of hydroxyapatite (HA), a layer that is responsible for the formation of a firm bond with hard and soft tissues (Kokobu *et al.* 2006). The ability of a material to form an HA-like surface layer when immersed in simulated body fluid (SBF) is often taken as an indication of its bioactivity. This *in vitro* bioactivity is an indication of the bioactive potential of a material *in vivo* (Rahaman *et al.* 2011).

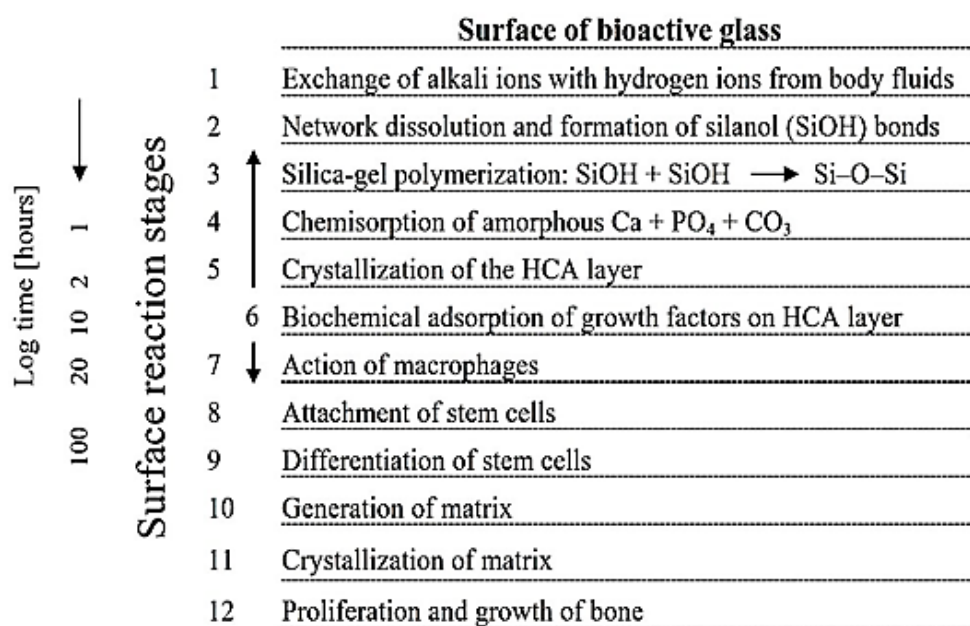
Bioactive glass is mainly composed of silicon dioxide, sodium oxide, calcium oxide and phosphorous pentoxide which are physiological chemicals found in the body. The bone-bonding reaction results from a series of reactions between the glass and its surface. (Hench and Andersson, 1993). When bioactive glass granules are inserted into bone defects, ions are released in the body fluids and precipitate into a bone-like apatite at the granule surface which promotes the adhesion and proliferation of osteogenic cells. The material will, soon thereafter, be replaced by new bone. The first step of the glass/solution interactions can be summarized as follow:

**1) Leaching and formation of silanols:** The glass network releases alkali elements cations such as  $\text{Ca}^{+2}$ ,  $\text{Na}^{+}$ ,  $\text{K}^{+}$ , etc. The exchange of cations releasing of glass occurs with  $\text{H}^{+}$  or  $\text{H}_3\text{O}^{+}$  cations proceeding from the solution makes the solution more alkaline resulting in higher value of interfacial pH, usually more than 7.4.

**2) Dissolution of the glass network:** The breakage of  $\text{--Si-O-Si-O-Si--}$  bonds through the action of hydroxyl ions leads to dissolution of glass network. This Breakdown of the silica network releases silic-acid  $[\text{Si}(\text{OH})_4]$ . The rate of dissolution of silica highly depends on glass composition. Presence of more than 60% of silica in glass decreases the dissolution rate due to the larger number of bridging oxygen bonds in the glass structure. The hydrated silica ( $\text{SiOH}$ ) formed on the glass surface by these reactions undergoes rearrangement by polycondensation of neighbouring silanols, resulting in a silica rich gel layer.

**3) Precipitation:** Calcium and phosphate ions are released from the glass together with those already present in the solution, form a calcium-phosphate rich layer (CaP) at the material surface. This calcium-phosphate layer is initially amorphous and later on crystallizes into hydroxycarbonate apatite (HCA). The mechanism of nucleation and growth of HCA appears to be the same *in-vivo* and *in vitro* and is accelerated by the presence of hydrated silica.

The precipitation of the HCA layer is then followed by reaction between the new formed reactive layer and the biological environment as shown in Figure 1. The HCA formation is followed by biochemical adsorption and desorption of growth factors and macrophages required to prepare the implant site for tissue repair are activated. Then, attachment of stem cells and synchronized proliferation and differentiation of the cells, rapidly occurs on the surface of bioactive materials. After that, bioactive materials begin to produce various growth factors which stimulate cell division, mitosis, and production of extracellular matrix proteins. Finally, mineralization of matrix results in mature osteocytes. (Hench, 2013)



**Figure 1:** Sequence of interfacial reactions involved in forming a bond between bone and a bioactive glass (Gerhardt et al. 2010)

The advantage of bioactive glass is that it is possible to design the glass to get a controlled rate of degradation and bonding to the tissue. The rate of degradation should be tailored in order to match the speed of the tissue healing. It is noteworthy that tissue healing time can vary greatly based on the tissue to be healed, the age of the patient and the mechanical constraint at the surgical site post-surgery. (Hench and Andersson, 1993)

The major disadvantage of bioactive glass is its high modulus and brittle nature. The brittleness and low fracture toughness limits its application in the biomedical field. Low fracture toughness refers to ability to resist fracture when a crack is present. Thus, bioactive glasses cannot be used for load-bearing implants. Especially, the repair and regeneration of large bone defects at load-bearing anatomical sites such as limbs is a great challenge for bioactive glass (Gerhardt *et al.* 2010) Although, bioactive glass are strong enough to function in stress bearing sites in the head and mandible replacement, they cannot be used in orthopedics. This is because, bioactive glass cannot be easily contoured in the operating room and screws cannot be easily placed into bioactive glass blocks because they resist drilling and have a tendency to fragment during creation of screw holes. (Nandi *et al.* 2011)

Most commonly studied bioactive glasses types are silicate, phosphate and borate based bioactive glass. Brief description of all types of bioactive glass is discussed below:

#### **a) Silicate based bioactive glass**

The most widely investigated bioactive glass composition is the bioactive silicate “45S5 Bioglass®”, which has a composition (in wt. %) of 45% SiO<sub>2</sub>, 24.5% Na<sub>2</sub>O, 24.5% CaO, and 6% P<sub>2</sub>O<sub>5</sub>. The key compositional features that are responsible for the bioactivity of 45S5 glass is its low SiO<sub>2</sub> content while comparing to other more chemically durable silicate glasses with high Na<sub>2</sub>O and CaO content and high CaO/P<sub>2</sub>O<sub>5</sub> ratio. (Rahaman *et al.* 2011) They offer remarkable advantages as the inorganic components of composite scaffolds due to their high bioactivity index. Indeed, this glass is rated Class A bioactive material, i.e. it has the ability to bond to both soft and hard connective tissues. Although, 45S5 glass remains the gold standard for bioactive glass some limitations exist, limiting in some case their clinical use. For instance, the low Si content of this glass along with its high alkali and alkaline earth content leads to a silicate structure prone to crystallization upon heating. Thus this glass cannot be sintered into porous scaffolds without crystallization. (Rahaman *et al.* 2011) (Massera *et al.*, 2012)

Similarly, the use of “S53P4” bioactive glass (BonAlive Biomaterials Ltd., Turku, Finland), with the specific composition (in wt. %) of 53% SiO<sub>2</sub>, 4% P<sub>2</sub>O<sub>5</sub>, 23% Na<sub>2</sub>O, and 20% CaO is increasing in clinical practice in various bone graft applications and in treatment of osteomyelitis. S53P4 bioactive glass is indicated to facilitate and stimulate bone formation and bone defect healing and is also supposed to have an antibacterial effect in various applications. S53P4 bioactive glass is mostly used in a granular form (0.8–

3.15 mm). (Van Gestel *et al.* 2015). However as for the glass 45S5 the hot working domain is rather low whereas the crystallization rate is high at typical sintering temperature (Massera *et al.* 2012). The “13-93” bioactive glass is another silicate glass that is receiving more attention nowadays. This glass has a higher SiO<sub>2</sub> content (53 wt. %) than 45S5 Bioglass®. (Tournie *et al.* 2013) The higher Si along with lower Na content makes this glass promising for scaffold manufacturing. Furthermore, the structural changes lead to a slower dissolution rate *in vitro*, which could be beneficial for the healing of large defects in bones. (Massera *et al.* 2012).

#### **b) Phosphate based bioactive glass**

Phosphate based glasses were considered to dissolve rapidly in aqueous media. However, some studies have demonstrated that phosphate glass structure can be modified to reach dissolution rates similar to those of silicate glasses (Bunker *et al.* 1984). Phosphate glasses can be used in various applications such as nuclear waste management (Day, *et al.* 1998), hermetic seals (Brow *et al.* 1996) or high power lasers (Weber *et al.* 1990) by tailoring its dissolution rate. Phosphate based glasses have been found to be good alternatives to silicate glasses in many biomedical applications, such as bone repair and reconstruction (Clement *et al.* 1990 & Knowles *et al.* 2003) Phosphate glass fibers for medical applications have been drawn by melt processing and have been found promising in soft tissue engineering applications. (Massera *et al.* 2014)

Recently a phosphate bioactive glass containing strontium and labelled SR50 was developed. (Massera *et al.* 2013) Physiologically, strontium and calcium are similar. They are both absorbed in the gastrointestinal tract, concentrated in bone and excreted primarily in urine. The mechanisms of strontium incorporation into bone, involves ionic exchange with bone calcium. Strontium is currently used in bone therapies. Strontium ranelate (SR) is marketed under the name of Protelos, Osseor, Protos, Bivalos, Protaxos and Ossum, and used to treat osteoporosis. It has been shown that SR strengthens bone, increases bone mass and density and lessens the possibility of vertebral and hip fracture in elderly women. (Abou Neel *et al.* 2009).

Phosphate glasses have the advantage over silicate bioactive glasses to have a congruent dissolution. Indeed, as mentioned earlier the dissolution of bioactive silicate glasses is primarily governed by the leaching of alkaline and alkaline earth ions within the solution while the Si is leached at lower rate forming a Si-rich gel. In the case of phosphate bioactive glasses, all ions in the glass network leach out at a similar rate and the surface of the glass does not change significantly its composition. As no gel layer is formed, the dissolution of phosphate based glass is more sustained over time (Khan *et al.* 2010) (Massera *et al.* 2014) (Bunker *et al.* 1984)

### c) Borate based bioactive glass

Recently, interest in borate glasses has increased largely due to very encouraging clinical results of healing of chronic wounds, such as diabetic ulcers, that would not heal under conventional treatment (Jones, 2012). The soft tissue response may be due to their fast dissolution, which is more rapid than that for silica based glasses. Borate bioactive glasses have shown to support cell proliferation and differentiation *in vitro* as well as tissue infiltration *in vivo* (Rahaman *et al.* 2011). Also, recent work has shown that by varying the composition of bioactive glass, its degradation rate can be controlled such as by partially replacing the  $\text{SiO}_2$  in silicate 45S5 glass with  $\text{B}_2\text{O}_3$ , the rate of degradation can be varied over a wide range. (Huang *et al.* 2006)

### 2.1.2 Calcium phosphate ceramics

Many implantations failed earlier because of infection as well as due to lack of knowledge about the toxicity of the selected materials. The use of calcium phosphates is reasonable due to their similarity to the mineral phase of bone and teeth and are easily accepted by human body. (Sakka *et al.* 2013). Calcium phosphate based biomaterials and bioceramics are now used in a number of different applications throughout the body, covering all areas of the skeleton. The different forms of commercially available calcium phosphates currently used in the biomedical industry are hydroxyapatite,  $\beta$ -tricalcium phosphate ( $\beta$ -TCP),  $\alpha$ -tricalcium phosphate ( $\alpha$ -TCP), biphasic calcium phosphate, monocalcium phosphate monohydrate and unsintered apatite. (Nor *et al.* 2009)

$\beta$ -tricalcium phosphate ( $\beta$ -TCP) is used in various orthopedic applications due to its osteoconductive and biodegradable nature. Although  $\beta$ -TCP has several favorable properties, it cannot be used in many loadbearing applications because of its poor mechanical properties (brittle in nature) and poor fatigue resistance. (Sakka *et al.* 2013) Hence,  $\beta$ -TCP have been combined with other calcium phosphate like HA in order to improve its mechanical property. HA is very stable but has low osteoconductivity. Combination of both develops biphasic calcium phosphate (BCP) which is supposed to overcome the limitations of each material. Several studies have demonstrated that BCPs can be used as bone substitutes successfully. (Lee *et al.* 2015)

## 2.2 Biodegradable polymer

Biodegradable polymers are the polymers that are degraded *in vivo* either enzymatically or non-enzymatically. By-products produced by such polymers are biocompatible and toxicologically safe which are further eliminated by the normal metabolic pathways (Makadia and Siegel, 2011). The American Society of Testing and Materials (ASTM) defines 'biodegradability' as: "capable of undergoing decomposition into carbon dioxide,

methane, water, inorganic compounds, or biomass in which the predominant mechanism is the enzymatic action of microorganisms, that can be measured by standardized tests in a specified period of time, reflecting available disposal conditions."

Biodegradable polymers have been used in different implants, eliminating the need of additional surgery to remove the implant. They are also supposed to be ideal for drug delivery application in which the implant should degrade and disappear over time. Since they are biocompatible, non-toxic in nature and its degradation rate can be controlled, they have been used extensively in the biomedical field. Beside this, they can also be fabricated into various shapes with desired pore morphologic features favorable to tissue in-growth. Furthermore, biodegradable polymers can be designed with specific chemical functional groups that can induce tissue in-growth. (Gunatillake & Adhikari, 2003) The basic category of biodegradable polymers used in biomedical field can be broadly classified as natural biodegradable polymers and synthetic biodegradable polymers.

- a) **Natural polymers:** Natural polymers are formed in nature during the growth cycles of all organisms. Natural biodegradable polymers are called biopolymers. The two main renewable sources of biopolymers are polysaccharides and proteins. Polysaccharides such as starch and cellulose, represent the most important family of these natural polymers. Natural polymers are often chemically modified in order to improve the mechanical properties as well as to modify their degradation rate. (Vroman & Tighzert, 2009) Since natural polymers are mostly derived from an animal source, the material purity of polymer can often differ from one batch to another. Furthermore, there is always a risk of transfer of disease from animal to human (Nair & Laurencin 2007).
- b) **Synthetic polymers:** Synthetic polymers are produced from non-renewable petroleum resources. Biodegradation of polymeric biomaterials involves cleavage of hydrolytically or enzymatically sensitive bonds in the polymer leading to polymer erosion. A large number of biodegradable polymers have been synthesized recently and some microorganisms and enzymes capable of degrading them have been identified. (Ghanbarzadeh & Almasi, 2013) Synthesis of synthetic polymers can be easily manipulated and the material impurities can be controlled. As a result, the mechanical and physical properties of synthetic polymers can be correctly predicted and reproduced. (Chen *et al.* 2008).

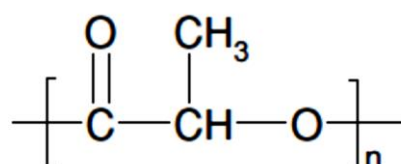
The most widely utilized biodegradable synthetic polymer for 3D scaffolds in tissue engineering are saturated aliphatic polyesters, typically poly- $\alpha$ -hydroxy esters such as poly lactic acid (PLA), poly glycolic acid (PGA), poly  $\epsilon$ -caprolactone (PCL) and their copolymers. The chemical properties of these polymers allow hydrolytic degradation through de-esterification. After degradation, the lactic and glycolic acid monomers are metabolized naturally by tissues. Due to these properties, PLA, PGA, PCL, and their copolymers



have successfully been applied in a number of biomedical devices, such as degradable sutures and bone internal fixation devices (BiofixW, Bionx Implants Ltd., Tampere, Finland) which have been approved by the US Food and Drug administration. (Chen *et al.* 2012)

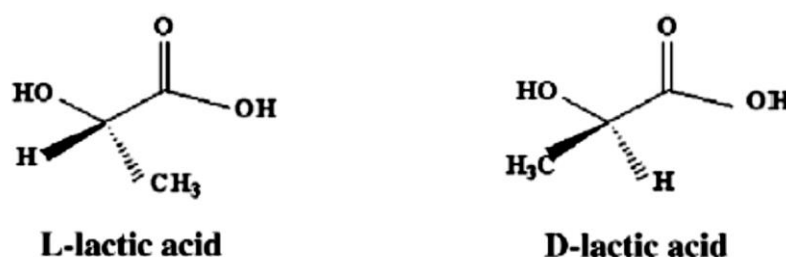
### 2.2.1 Poly lactic Acid (PLA)

Poly lactic acid belongs to the family of aliphatic polyesters commonly made from acid, and are considered as biodegradable and compostable. It is a synthetic biodegradable polyester where the monomer is lactic acid (LA). LA is derived from natural resource which is produced by bacterial fermentation of carbohydrates such as corn, sugarcane, potatoes and other biomass. (Lim *et al.* 2007) The chemical structure of Poly lactic acid (PLA) is shown in Figure 2.



**Figure 2:** Chemical Structure of PLA (Averous & Pollet, 2012)

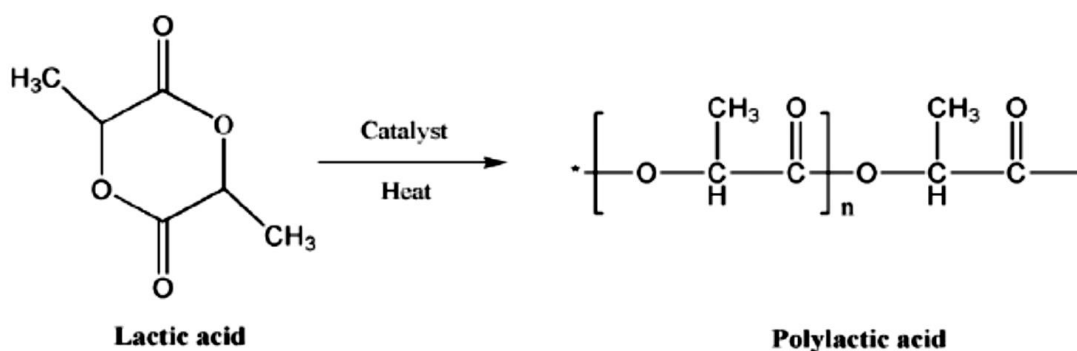
PLA can exist in two optically active stereoisomeric forms, L-lactic acid and D-lactic acid as shown in Figure 3. L-lactic acid is a naturally occurring isomer. Polymers of L-lactic acid and D-lactic acid are called PLLA and PDLA respectively (Averous and Pollet, 2012). PLA can be either amorphous or semicrystalline, depending upon its stereochemistry and thermal properties. PLA can be produced totally amorphous or up to 40 % crystalline. PLA resins containing more than 93 % of L-lactic acid are semi-crystalline while PLA with 50–93 % L-lactic acid is completely amorphous. (Henton *et al.* 2005) Therefore, the properties of poly lactic acid, such as melting temperature and crystallinity are highly dependent upon ratio of L and D enantiomers (Carrasso *et al.* 2010)



**Figure 3:** Isomers of Lactic acid (Nampoothiri *et al.* 2010)

Commercially available PLA is PDLDA, which is a copolymers of poly (L-lactic acid) (PLLA) and poly (D, L-lactic acid) (PDLA). Poly (L-lactide) is characterized by a high content of crystals, high strength particles, and prolonged period of degradation. In contrast, Poly (DL lactide) is characterized by lower strength and quicker rates of degradation. PDLDA has a structure that combines the best characteristics of poly (L-lactic acid) and poly (D-lactic acid that is, the mechanical properties of the poly(L-lactide) and the shorter degradation time of the poly (DL-lactic acid). These properties have made PLDLA a compound of great relevance in the controlled release of drugs and in bone tissue engineering. (Masa *et al.* 2015)

PLA can be synthesized using three different routes: direct condensation polymerization, azeotropic dehydrative condensation, and ring-opening polymerization of lactide. Generally, PLA produced by these methods are of high molecular weight. Among these three methods most commonly used method is ring opening polymerization as shown in Figure 4. Direct condensation polymerization is the least expensive method, but only low molecular weight PLA can be produced due to difficulties in removing water. (Jiang & Zhang, 2013)



**Figure 4:** Synthesis of PLA by ROP (Jiang & Zhang, 2013)

PLA can be considered as a polymer with the broadest range of applications because of its ability to be stress crystallized, thermally crystallized, impact modified, filled, copolymerized, and processed in most polymer processing equipment. (Henton et al 2005). PLA can be processed into fiber, film, sheet, and 3D articles using different processing technologies such as drying, extrusion, injection molding, injection stretch blow molding, casting, blown film, thermoforming, foaming, blending, fiber spinning etc. (Jiang & Zhang, 2013)

The typical glass transition temperature ( $T_g$ ) of PLA ranges from 50 to 80 °C while the melting temperature( $T_m$ ) ranges from 130 to 180 °C. For example, for enantiomerically pure PLA,  $T_g$  is 55 °C and  $T_m$  is 180 °C. For such semi-crystalline PLA, the  $T_m$  is a function of the different processing parameters and the initial PLA structure.  $T_m$  increases

with the molecular weight ( $M_w$ ) until a maximum value. Besides that, the crystallinity decreases with increasing  $M_w$ . (Averous and Pollet 2012) The  $T_g$  and  $T_m$  of PLA are strongly affected by overall optical composition, primary structure, thermal history and molecular weight. Amorphous PLAs transfer from glassy to rubbery, above  $T_g$  and upon further heating, it will behave as a viscous fluid. While below  $T_g$ , PLA behaves as a glass with the ability to creep until cooled to its  $\beta$  transition temperature of approximately  $-45^\circ\text{C}$ . Below this  $\beta$  transition temperature, PLA will only behave as a brittle polymer. (Henton *et al.* 2005)

PLA readily absorbs moisture from the atmosphere as it is hygroscopic thermoplastic. The presence of even small amounts of moisture will hydrolyze PLA in the melt phase, reducing the molecular weight. As a result, the mechanical property of PLA is decreased. Therefore, PLA must be thoroughly dried prior to melt processing. PLA is generally dried at temperature range of  $80\text{--}100^\circ\text{C}$ . Excessive heat and moisture should be avoided during processing of polymer which may lead to thermal and hydrolytic degradation. (Lima *et al.* 2008)

Most potential applications of PLAs in the medical fields are tissue engineering, wound management, drugs delivery, and orthopedic devices. Different types of biodegradable screws, fixation pins, plates, and suture anchors have been used as orthopedic device. (Hamad *et al.* 2014) However, most common biomedical application of PLA is Dexton fibre which is used as resorbable suture. Fibers can be prepared by either melt spinning or solvents pinning method. Fiber prepared by solvent spinning has higher mechanical properties in comparison to melt spinning where there are chances of thermal degradation (Södergård and Stolt, 2002). PLA is very stable and will retain its molecular weight and physical properties for years. Thus, its use in clothing and durable applications is increasing. Also, high molecular weight PLA is also naturally resistant to supporting bacterial and fungal growth, which allows it to be safely used for applications such as food packaging and sanitation. (Nampoothiri *et al.* 2010).

### **2.2.2 Hydrolytic behavior of Polylactic acid (PLA)**

The degradation mechanism of aliphatic polyesters like PLA, PGA and their copolymers is hydrolysis. The chemical structure of such polymers consists carbon-oxygen-carbon (C-O-C) bonds in their polymer chain. When such polymer are exposed to water, the water molecules react with C-O-C bond resulting in breakage of bond. (Borden 2006) However, the degradation mechanism of aliphatic polymers has been found to be dependent on a large number of factors, such as molecular weight, crystallinity, purity, temperature, pH, presence of terminal carboxyl or hydroxyl groups, water permeability, and additives acting catalytically that may include enzymes, bacteria or inorganic fillers (Park and Xanthos, 2009, Averous, 2008).

For example, crystallinity plays important role in the hydrolysis rate of polymer. The crystalline segments in the polymer are more stable than the amorphous segments which are responsible for slowing down the hydrolysis rate of polymer. Similarly, polymer with longer polymer chains with high molecular weights degrades slowly than those with shorter chain with lower molecular weights. Also, chemical structure and composition of polymer play important. Some polymer consists of bond that are susceptible to hydrolysis than other as well as some groups in polymer backbone which can be either hydrophilic or hydrophobic in nature that directly effects on the degradation phenomenon of polymer. For instance, PLA consist of methyl group in the backbone which is hydrophobic in nature. Thus PLA degrades more slowly than other aliphatic polymer like PGA. (Li, 1999, Middleton & Tipton 2000, Borden 2006, Rezwan et al.2006)

Poly lactides undergo hydrolytic degradation via the bulk erosion mechanism by the random scission of the ester backbone. It degrades into lactic acid, a normal human metabolic byproduct, which is broken down into water and carbon dioxide via the citric acid cycle. The degradation mechanism of PLA starts with hydrolysis, followed by bacterial attack on the fragmented residues. The rate of hydrolysis is accelerated by acids or bases and is dependent on moisture content and temperature. (Nampoothiri *et al.*2010)

The hydrolytic degradation of PLA can be divided into two stages. At first, the water diffuses into the amorphous regions of the polymer with random hydrolytic scission of ester bond, which converts the long polymer chains into shorter ones. Because this occurs in the amorphous regions there is a reduction in molecular weight but no loss in physical properties, while the crystalline regions hold the structure together. In the second stage the crystalline regions fragment and the physical properties diminish. Later, the fragments are metabolized by enzymes resulting in a rapid loss of polymer mass. (Jiang & Zhang, 2013, Nair & Laurencin, 2007)

## 2.3 Composite

Composite can be defined as a multiphase material made from a combination of materials, differing in composition, which remain bonded together, but retain their identities and properties, without going into any chemical reactions. (Agrawal, 1990) Some of the unique advantages of composites are its high strength, high stiffness, long fatigue life, low density, and adaptability to the intended function of the structure. Also, composites materials can be easily moulded into any complex shape. (Agrawal, 1990) Bone is a simple example of a natural composite material having the best properties of its constituents. Bone must be strong and rigid but yet flexible enough to resist breaking under normal use. These essential properties are contributed by its components. A mature bone is made up of two basic kinds of materials, organic and inorganic. The organic component, consisting mostly of proteins, carbohydrates and fats, makes it pliable and gives the required

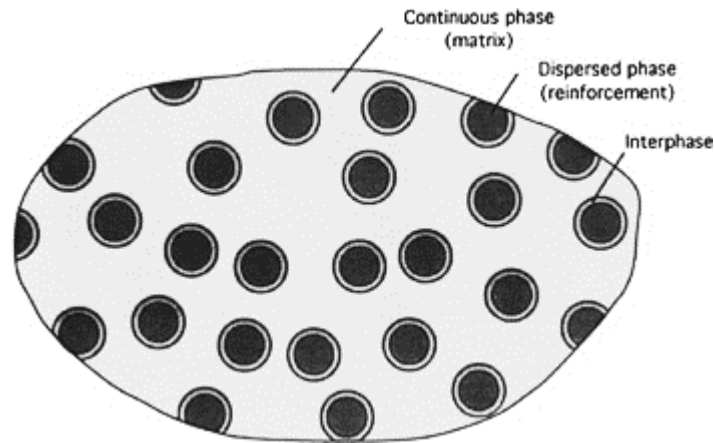
softness. The inorganic consists mainly of hydroxyapatite (HA) that maintains the mechanical strength. (Agrawal, 1990)

Different material has its identical properties with both advantage and disadvantage. For examples: plastics are light, durable, have excellent corrosion resistance and can be easily molded to any complex shape but they lack sufficient strength, stiffness and dimensional stability. (Harris, 1999) Similarly, ceramics have great thermal stability and are resistant to most forms of attack such as abrasion, wear and corrosion. Although ceramics are intrinsically very rigid and strong because of their chemical bonding, they are all brittle and can be formed and shaped only with difficulty. (Harris, 1999) Hence, different materials can be combined to form a composite material with optimum properties. The mechanical, biological and physiological properties of such composite can be tailored according to required application and are supposed to be superior and unique while comparing the properties of individual component. (Huang and Ramakrishna, 2004)

For an example, although calcium phosphates ceramics are one of the established materials for the augmentation of bone defects, they exhibit relatively poor tensile and shear properties. In practice, the strength of the calcium phosphate cements is lower than that of bone, teeth, or sintered calcium phosphate bioceramics and are generally brittle in nature. Hence, calcium phosphate ceramics can be combined with different biodegradable polymer to form a composite in order to not only improve the mechanical properties but also favor bone healing. (Sakka *et al.* 2002).

### **Classification of composite**

Composite material is a multi-phase system whose internal structure consists of three basic physical phases called matrix phase, reinforcement phase and composites interface phase, as shown in Figure 5. (Daniel and Ishai, 1994). Composite interface phase is the interface between the reinforcement phase and the matrix phase. The performance of a composite materials is determined by the structure and the nature of these phases, their configuration and interaction. (Wang *et al.* 2011) Matrix phase is primary phase having a continuous character called matrix. It is usually more ductile and weaker phase that holds the dispersed phase and shares a load with it. Matrix material includes metal matrix composite materials, inorganic non-metallic matrix composite materials and polymer matrix composites by the different matrix materials. Dispersed phase is the secondary phase that is embedded in the matrix in a discontinuous form. Dispersed phase is scattered and surrounded by the matrix and is usually stronger and stiffer than the matrix phase. A dispersed phase usually includes fibrous materials such as glass fiber, organic fiber. The interphase also plays an important role in controlling the failure mechanisms, fracture toughness, and overall stress–strain behavior of the material. Hence, the properties of a composite material depend on the properties of the constituents, geometry, and distribution of the phase. (Harris, 1999, Daniel and Ishai, 1994) The three different phases of composite are represented in Figure 5 below. (Daniel and Ishai, 1994)



**Figure 5:** *Phases of composite material (Daniel and Ishai, 1994)*

According to the matrix phase, composite is classified as: polymer-matrix composites (PMC), metal-matrix composites (MMC), ceramic-matrix composites (CMC) and carbon-carbon matrix composites (CCM). Composites can also be classified according to the dispersed (reinforcing) phase as particle reinforced composites, short-fiber reinforced composites and long-fiber reinforced composites. Long-fiber reinforced composite is further classified as unidirectional and woven lamina. (Daniel and Ishai, 1994) In biomedical application, the most commonly used is polymer matrix composites (Huang and Ramakrishna, 2004)

Polymer matrix composite material is the one that uses organic polymer as matrix and fiber as reinforcement. Normally, the strength and modulus of fiber are much higher than the matrix material which makes it the main load-bearing component. In addition, matrix material is supposed to have good adhesion properties in order to bond fibers together firmly. At the same time, the matrix material can serve to uniformly distribute the applied load, and transfer the loads to fiber. The properties of composite materials is mainly dependent on the characteristics of the matrix material. As a result, the performance of composite materials is directly influenced by the performance of fiber, matrix and the interface between them. (Wang *et al.* 2011) Polymer matrix composites include thermoset like epoxy, polyimide and polyester, thermoplastic like poly-ether-ether-ketone and polysulfone which are reinforced with glass, carbon (graphite), aramid (Kevlar), or boron fibers. They are used primarily in relatively low temperature applications. (Daniel and Ishai, 1994)

## **2.4 Composite of glass and polymer**

Glasses have high chemical stability, but they are likely to lose their mechanical strength at relatively low temperatures as they pass through the glass transition. The principal problem with glassy materials is that they are always brittle and their measured strengths

are very variable at ordinary temperatures. Few Polymers are thermally stable in comparison with metals or ceramics. Major disadvantage of polymers is their very low mechanical strength and stiffness in bulk form. (Harris, 1999)

Various studies have been made in order to compare the property of glass and polymer composite in terms of bioactivity and mechanical properties (bending, compression, tensile, shear strength and elastic modulus) as a function of degradation time. Bioactive glasses have been combined with different biodegradable poly ( $\alpha$ -hydroxyacids) like bioresorbable PLA, poly (glycolic acid) (PGA), and their copolymer poly (lactic acid-co-glycolic acid) (PLGA). (Langer & Vacanti 1993, Maquet & Jerome 1997, Stamboulis *et al.* 2001 Sherwood *et al.* 2002, Blaker *et al.* 2003, Maquet *et al.* 2003, Maquet *et al.* 2004, Lu *et al.* 2002, Li *et al.* 2005, Niemelä *et al.* 2005, Niemelä *et al.* 2008, Niiranen *et al.* 2004, Paivaa *et al.* 2006, Lehtonen *et al.* 2012, Gough *et al.* 2003, Roether *et al.* 2001). The combination of such polymers with a bioactive component takes advantage of the osteoconductive properties of bioactive glasses and of their strengthening effect on polymer matrices. The composite is expected to have superior mechanical properties than the neat unreinforced polymer and to improve structural integrity and flexibility over brittle glasses for eventual load-bearing applications. (Maquet *et al.* 2004) In addition, several studies have also been made on  $\beta$ -tricalcium phosphate ( $\beta$ -TCP) and biodegradable polymer composites. (Niemela *et al.* 2008, Ahola *et al.* 2013).

#### **2.4.1 Porous bioactive glass/polymer composites**

Maquet *et al.* (2003, 2004) studied the in vitro study of highly porous poly (D, L-lactide)/ Bioglass® composites scaffolds and porous poly ( $\alpha$ -hydroxyacid)/ Bioglass® composite foam that were prepared by freeze-drying. Two series of composite scaffolds made of PDLLA and PLGA were prepared by adding different amounts of the Bioglass® (10, 25 and 50 wt. %) in the mixture. In vitro degradation test was done for 78 days in Phosphate based buffer (PBS). It was observed that the water absorption was increased with the increase in Bioglass® content. The PLGA/ Bioglass® composites adsorbed larger amount of water (~600%) than PDLLA/Bioglass® composites which confirmed that the PLGA based composite are more hydrophilic as compared to PDLLA based composite. It was found that the compressive modulus of the composites was significantly improved by the Bioglass®. The compression modulus of both PDLLA/Bioglass® and PLGA/Bioglass® composites foams was significantly higher than that of the neat polymer foams. The polymer molecular weight, determined by size exclusion chromatography, was found to decrease more rapidly and to a larger extent in absence of Bioglass®. The presence of the bioactive filler found to delay the degradation rate of the polymer as compared to the neat polymer foams. The formation of HA on the surface of polymer/ Bioglass® composites was confirmed by both X-ray diffraction and Raman spectroscopy. (Maquet *et al.* 2003, 2004). Similar types of results have been reported by Bocaccini and Maquet (2003).

Similarly, Lu *et al.* studied the porous composite of polylactide-co-glycolide (PLGA) and 45S5 bioactive glass (BG) that is biodegradable, bioactive, and suitable as a scaffold composite. Structural and mechanical properties of PLGA-BG were found to be better than PLGA alone. The addition of bioactive glass granules to the PLGA matrix resulted in a structure with higher compressive modulus than PLGA alone. The PLGA-BG composite was found to be a bioactive material, as it formed HA deposits at its surface when immersed in a simulated body fluid (SBF), and in the presence of cells and serum proteins. (Lu *et al.* 2002).

Also, Li *et al.* 2005 studied the porous polyhydroxybutyrate-polyhydroxyvalerate (PHBV)/bioactive glass (PHBV/BG) composite scaffolds that were prepared by compression molding, thermal processing, and particulate leaching method. The *in vitro* degradation test showed that water absorption was increased with addition of bioactive glass which resulted in more weight loss due to the dissolution of bioactive glass particles. Also, the addition of BG to the PHBV matrix resulted in a structure with significantly higher compressive yield strength than that of the pure PHBV. The rapid formation of apatite on composite scaffolds after 3 days of incubation in SBF indicated the high bioactivity of the composites. As it is known that the degradation rate of temporary scaffolds must be matched to the rate of new tissue formation for tissue engineering and tissue repair applications and this study shows the possibility to modulate the degradation rate of composite scaffolds by incorporation of BG into the polymer matrix.

### 2.4.2 Bioactive glass coated composites

Polymer/bioactive glass composites can also be prepared by coating the polymer with bioactive glass. Stamboulis *et al.* (2001), have studied the effect of bioactive glass coating on commercially available Polyglactin 910 (Vicryl®) sutures. Bioactive glass was coated by layer-pressing procedure. The resulted coating was not homogenous as expected. However, bioactive glass coating was supposed to alter the mechanical properties as well as it was expected to act as protective layer for polymer slowing down the degradation rate.

Similarly, Gough *et al.* (2003), studied the polymer/bioactive glass composite prepared by coating PDLA foams by Bioglass® by using aqueous slurry-dipping technique. The formation of crystalline HA was formed on the Bioglass® coated PDLA foams after 7 days of immersion in SBF. HA was also on the surfaces of non-coated PDLA foams, however the rate and amount of HA formation were much lower than in the composites. The rapid formation of HA on the Bioglass®/PDLA foam surfaces confirmed the high bioactivity of these materials. Also, Osteoblasts cell was found to be attached within the porous network throughout the depth of the foams. Cell density was found to be higher in the PDLA/ Bioglass® composites compared to the pure PDLA foams. Hence, it was confirmed that the composite foams developed exhibited the required bioactivity to be



used as scaffolds for bone tissue engineering. Similar study has been made by Roether *et al.* (2001).

### 2.4.3 Dense Polymer/bioactive glass composites

Paivaa *et al.* (2006) studied the *in vitro* behavior of Bioactive Glass/polyhydroxybutyrate Composite. The main aim of this work was to evaluate the *in vitro* bioactivity of bioactive composite. The *in vitro* tests were conducted for two different weight percentage of polymer/bioactive glass composites i) bioactive glass/PHB 30/70 wt. % and ii) bioactive glass/PHB 40/60 wt. % in SBF for 14 days. *In vitro* studies show that Bioglass®/PHB composites (30/70 wt. % and 40/60 wt. %) have formed a layer of Ca-P. It was suggested that these composites have enough bioactivity to be used as biomaterial. The composites Bioglass®/PHB 30/70 wt. % form a Ca-P rich layer faster than Bioglass®/PHB 40/60 wt. %. The Ca-P layer formed on Bioglass®/PHB 30/70 wt. % has a Ca/P ratio of approximately 1.67, which is the Ca/P ratio of HA. The quantity of bioactive glass and the size of particles influence the bioactivity of composite. It was observed that larger particles and high quantity of particles presented less bioactivity than composite with smaller particles and intermediate size particles. (Paivaa *et al.* 2006)

Niiranen *et al.* (2004), studied the self-reinforced bioabsorbable polymer/bioactive glass composites in a phosphate-buffered saline for 87 weeks. Addition of bioactive glass 13-93 on PLLDA, increased the hydrophilic nature of the composites by glass/matrix interfaces and promoting the macropores in the structure of the composites at die-drawing. However, the faster WA of the composites did not accelerate the degradation of the polymer matrix according to the decrease in molecular weight and the increase in crystallinity.

Similarly, Lehtonen *et al.* (2012) studied the *in vitro* degradation of three bioresorbable glass fiber-reinforced poly(L-lactide-co-DL-lactide) (PLDLA) composites in simulated body fluid (SBF and deionized water for 52 weeks. The degradation mechanism was found to be a combination of surface and bulk erosion and does not follow the typical core-accelerated degradation mechanism of poly( $\alpha$ -hydroxyacids). It was found that strength retention by bioresorbable glass fiber-reinforced PLDLA composites can be tailored by altering the the composition of the glass fibers. In addition, the strength retention properties of resorbable composites depends upon the selection of matrix type in terms of molecular weight and hydrophobicity as well as on processing method.

Niemelä, (2008), studied the *in vitro* behavior of self-reinforced composite of PLA matrix (Poly-L/DL-lactide 70/30 and Poly-L/DL-lactide 96/4) and spherical bioactive glass 13-

93(BaG) particles filler in Phosphate based buffer for 102 weeks. The cylindrical composite rod was prepared by twin-screw extruder. During the *in vitro* test series, it was observed that initial mechanical properties were affected by amount of filler. With an increase in filler content, the mechanical properties were found to decrease faster upon immersion in physiological medium. The sample proved to be bioactive if the BaG content was between 20-40% which have a large number of open pores in the surface for the rod like sample with enough CaP precipitation. It showed that bioactivity of sample depends upon number of open pores which depends upon filler content. Enough porosity was not observed in case of 10% and 50% containing BaG sample, hence they are not bioactive enough to be used in medical application. Also, Niemela found that the addition of osteoconductive filler in the polymer matrix increases the amount water absorbed by the sample because of porous structure of sample, more water could penetrate into the sample. This could be because of weak interlocking between BaG and polymer matrix as well as by higher porosity of sample containing BaG.

The addition of osteoconductive filler material like BaG maintained the pH of buffer solution for longer time. This could probably due to releasing of alkaline ion from BaG in the surrounding buffer which bind to the hydrogen ion from solution and thus acts as alkali, neutralizing the acidic degradation product of PLA resulting in constant pH for longer time. (Niemelä, 2008) Similar result has been observed by Lu *et al.* (2002) during the *in vitro* study of biodegradable polylactide-co-glycolide (PLAGA) and 45S5 bioactive glass (BG) composite. During the hydrolysis reactions, PLAGA degrades into glycolic and lactic acid, the release of which can cause a biologically significant decrease in local pH. Through dissolution reactions, BG releases alkaline ions, which produce an elevated local pH. By forming a composite of PLAGA and BG, the acidic byproducts produced during polymer degradation and the alkalinity due to the release of alkaline and alkaline earth ions maintained the physiological pH up to 3 weeks of culture.

#### **2.4.4 $\beta$ -Tricalcium Phosphate/biabsorbable polymers**

Niemela *et al.* (2005), studied the effect of  $\beta$ -tricalcium phosphate addition on the *in vitro* degradation of self-reinforced (SR) poly-L, D-lactide in PBS for 104 weeks. During the *in vitro* test series, it was observed that addition of  $\beta$ -TCP in polymer matrix affected the strength retention. The sample containing  $\beta$ -TCP retained their mechanical properties longer than plain matrix polymer. This could probably be due to better mechanical interlocking of  $\beta$ -TCP with polymer matrix. The initial mechanical properties of the SR-composites were lower compared to the SR polymer, but the degradation was slower in terms of mechanical properties, mass loss and molecular weight of the samples and the pH of the buffer solution.

Similarly, Ahola *et al.* (2013) also studied the *in vitro* behavior of composites of poly (L-lactide-co- $\epsilon$ -caprolactone) 70/30 and beta-tricalcium phosphate which were manufactured using extrusion to form biodegradable composites. The hydrolytic degradation of the composites containing 0, 10, 20, 35 and 50% of  $\beta$ -TCP was studied *in vitro* for 52 weeks in phosphate based buffer solution. During *in vitro* study, it was observed that  $\beta$ -TCP had a slight effect on the degradation properties which was mainly seen in the water absorption behaviour, as  $\beta$ -TCP dissolution was very slow in comparison with the copolymer degradation. However, mass loss and water absorption behaviour were significantly changed at 12 weeks *in vitro*. It was found that the mass loss of the plain copolymer was fastest while the mass loss of the composite containing 50% of  $\beta$ -TCP was slowest. This was attributed to the fastest dissolution rate of the polymer compared to  $\beta$ -TCP. The authors also observed that at short immersion time (less than 12 weeks) the composite containing the most  $\beta$ -TCP exhibits the highest water uptake. This was attributed to the hydrophilicity of the ceramics particles. However, at longer immersion time, it was assumed that the degradation of the polymer through chain scission led to a polymer more prone to absorbed water than the  $\beta$ -TCP. Thus was thought to explain the reason for increased water uptake in the plain polymer and low ceramic containing composite.

During the *in vitro* test, Ahola *et al.* (2013) found that the pH of the buffer was very stable in the first weeks of the test series. After 10 weeks, pH values were decreased which may be due to the acidic degradation products of the polymer degradation that were released to the hydrolysis medium. Throughout the *in vitro* test series, a tendency of increasing pH value towards the composites with higher  $\beta$ -TCP contents were observed. This was likely due to the release of Ca from the ceramic particles.

### 3. MATERIALS AND METHODS

#### 3.1 Preparation of glass/polymer composite

Medical grade PLDLA 70/30, batch number D150400013 with inherent viscosity 4.10 (dl/g) was obtained from Boehringer Ingelheim Pharma KG, that was supplied by Evonik Nutrition & Care GmbH, Darmstadt, Germany. Both Sr50 and 13-93 bioactive glass powder of size 125-250  $\mu\text{m}$  were prepared in laboratory. Glass 13-93 was melted from batches containing mixtures of sand (99.4 % pure  $\text{SiO}_2$ ), and analytical grades of  $\text{Na}_2\text{CO}_3$ ,  $\text{H}_3\text{BO}_3$ ,  $\text{CaCO}_3$ ,  $\text{K}_2\text{CO}_3$  and  $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ . The glasses were melted in air in a platinum crucible at temperature 1400°C. Glass Sr50 was melted from batches containing  $\text{NaPO}_3$ ,  $\text{Ca}(\text{PO}_3)_2$  and  $\text{Sr}(\text{PO}_3)_2$ . The composition of 13-93 bioactive glass and Sr50 bioactive glass are presented in Table 1 below.

*Table 1: Showing composition of bioactive glasses*

Glass type	Name	Composition
Silicate	13-93	$53\text{SiO}_2\text{-}6\text{Na}_2\text{O}\text{-}2\text{K}_2\text{O}\text{-}5\text{MgO}\text{-}\text{CaO}\text{-}4\text{P}_2\text{O}_5$
Phosphate	Sr50	$50\text{P}_2\text{O}_5\text{-}10\text{Na}_2\text{O}\text{-}20\text{CaO}\text{-}20\text{SrO}$ .

Before melt extrusion, both glass and PLA was dried thoroughly for 8 hours at 80 °C in vacuum. The polymer material should be dried in order to make it free from moisture as presence of moisture may lead to the polymer hydrolytic degradation.

Dried semi-crystalline PLA polymer and 13-93 powder (125-250  $\mu\text{m}$ ) were processed into rod-shaped composite with a diameter of approximately 2.2 mm with a co-rotating custom-built intermeshing twin-screw extruder (Mini ZE, 20\*11.5 D, Neste Oy, Porvoo, Finland) under nitrogen atmosphere. Total amount of polymer used was 600 grams and total amount of glass used was 100 grams. The feed rate for polymer and glass was varied to obtain 10, 30 and 50 wt. % of glass in the polymer. To maintain the glass-containing polymer melt viscosity constant the temperature of the extruder was varied depending on the glass content as shown in Table 2.

*Table 2: Extrusion temperatures and weight for preparing polymer/glass composites*

Sample name	Temperature (°C) Zone 1	Temperature (°C) Zone 2	Temperature (°C) Zone 3	Temperature (°C) Zone 4	Feed rate of polymer g/h	Feed rate of glass g/h
<b>PLA</b>	180	178	197	203	100	-
<b>PLA+10% 13-93</b>	174	172	191	197	108	12
<b>PLA+30% 13-93</b>	171	169	188	195	126	54
<b>PLA+50% 13-93</b>	171	169	188	195	150	50
<b>PLA+10% SR50</b>	186	180	201	206	108	12
<b>PLA+25% SR50</b>	197	193	212	217	126	54
<b>PLA+35% SR50</b>	194	192	209	214	150	50

PLA and glass were fed with separate gravimetric screw feeders and the mixing of the components took place in the extruder. A caterpillar belt was used to guide the composite rods from the die and the diameter of the composite rod was controlled by adjusting the speed of the belt. The speed of the belt was set to 10 meters per minute. The composites containing silicate glass are denoted as PLA+ 10% 13-93, PLA + 30% 13-93, PLA + 50% 13-93. Similarly, other types of composites containing phosphate glass were denoted as PLA+ 10% SR50, PLA + 25% SR50, PLA + 35% SR50. The approximate diameter of composite rod obtained was 2.2 mm. The composite rod was cut at 1-meter length for each and at least 6 meters of composite rod was selected for each composite. The rods were then cut to 70 mm length for hydrolysis test.

### 3.2 Preparation of TRIS Buffer

To prepare the TRIS buffer, 400 ml of distilled was taken in a beaker and was left to stir with magnetic stirrer for 15 minutes. Then, required amount of TRIS HCL and TRIS base as mentioned in table 3 was added slowly to the solution. The solution was allowed to stir

for 15-20 minutes until it become homogenous. When, the solution become homogenous, the beaker was removed from stirrer and was kept in water bath set to 37°C for an hour. Then, the pH of the solution was measured by a pH meter (Mettler-Toledo GmbH, Schwzebbach, Switzerland). pH meter was calibrated before measuring the pH. And the measured pH of solution was between 7.35-7.39 at 37° C. The prepared solution was transferred to a 1 litre round bottom flask and 600 ml of distilled water was added. TRIS buffer was always stored in freezer before use.

**Table 3:** Chemical used to prepare TRIS

Reagent	Amount (mg)	Manufacturer
Trizma Base	1.66	Sigma Aldrich
Triszma Acid	5.70	Sigma Aldrich

### 3.3 *In vitro* Hydrolytic test

For *in vitro* test, the estimated length of the sample was 70 mm. For each test point, 3 parallel samples were used. All the samples were weighted before immersion test to get the initial dry weight of the samples. The amount of TRIS buffer was calculated according to International Standard ISO 15814 for *in vitro* degradation testing (volume/weight ratio was greater than 30:1 ml/g). As average weight of our sample was 0.4 g, the estimated amount of buffer to be used in *in vitro* test was 12 ml. and the immersed samples were placed in shaking incubator at 37°C. The buffer was changed every two weeks. The *in vitro* test was done for 24 h, 48 h, 72 h, 1 wk, 2 wk, 4 wk and 6 wk. Test samples were withdrawn at predetermined time point. At each time point, pH measurement, wet weight measurement, dry weight measurement, mechanical testing (bending and shear), Gel Permeation Chromotography (GPC), FTIR and AAS were carried out.

### 3.4 Differential Thermal Analysis

The glass content of the test samples was measured using Differential Thermal Analysis (NETZSCH, Leading Thermal Analysis, STA 449F1). All tests were performed in alumina (Al<sub>2</sub>O<sub>3</sub>) crucible and in N<sub>2</sub> atmosphere. Approximately, 20 mg of sample was used and samples were heated from 25°C up to 1000°C. The heating rate was 10°C/min. The results were analyzed by using Proteus Analysis software. Two parallel samples were analyzed for each type of polymer/glass composition.

### 3.5 Mass loss and water absorption

The dry weight of each samples was taken prior to immersion test. At each time points, the samples were carefully wiped with tissue to remove excess water. Each of the test

samples were weighed immediately after wiping in order to obtain the wet weight of sample. Successively, the samples were cleaned with ethanol to dry the sample and stop the hydrolysis reaction and the samples were allowed to dry for one week in vacuum. After vacuum drying, each samples were weighed again to obtain their dry mass. Dried test samples were stored in a desiccator in view of their mechanical testing.

The mass loss was calculated as the difference between the mass of the initial test sample and the mass of the dried test sample divided by the initial mass of the test sample. Similarly, the water absorption (WA) was calculated as the difference between the mass of the wet test sample and the mass of the dried test sample divided by the mass of the dried test sample.

$$\text{Mass Loss (\%)} = \frac{\text{dry weight} - \text{initial weight}}{\text{initial weight}} \times 100\% \text{ (i)}$$

$$\text{WA (\%)} = \frac{\text{wet weight} - \text{dry weight}}{\text{dry weight}} \times 100\% \text{ (ii)}$$

### 3.6 Mechanical testing (bending and shear test)

The mechanical testing was done for all composite. All tests were done in triplicate. The mechanical testing was conducting prior and after immersion test. The purpose of the test was to ascertain the initial mechanical properties of composites as a function of glass composition and glass content, as well as to as a function of immersion time. The dry samples were mechanically tested at room temperature by three-point bending and shearing using Instron 4411 mechanical testing apparatus (Instron Ltd. High Wycombe, England).

The three-point bending test was done according to the standard SFS-EN ISO 178 Plastics - Determination of flexural properties. (SFS-EN ISO 178 2011). The diameter of the test samples was measured with a slide gauge. And shear test was done using Standard BS 2782 method 340B (1978). After mechanical testing, all the sample pieces were collected and placed in dessicator for further analysis. The average values and standard deviations were calculated. All samples were measured in triplicate. Parameter used at three-point bending and shear test can be seen in table 4 below:

**Table 4:** Parameters for 3 point bending and shear test

Parameter	Bending	Shear
Load cell	500 N	500 N
Crosshead speed	5 mm/min	10 mm/min
Radius (loading edge)	1.5 mm	-
Length (bending span)	32 mm	-

### 3.7 Gel Permeation Chromatography

For GPC analysis dry samples were used. The estimated weight for GPC sample was calculated to be  $7.35 \pm 0.15$  mg for the plain polymer. The mass of sample was adjusted taking into consideration the mass of glass presents in the sample to be studied. The samples were dissolved overnight in 5ml of chloroform (chromasolv for HPCL,  $\geq 99.8\%$ ) obtained from Sigma Aldrich and glass particle is filtered in order to analyze the molecular weight of polymer only. HPLC Filter (GHP Acrodisc 25 mm Syringe Filter with 0.2 micrometer GHP membrane) obtained from Life sciences were used during the GPC process.

### 3.8 Atomic Absorption Spectrometry

For Atomic absorption spectrometry (AAS) test, 5 ml of the immersing solution was collected and diluted to 50 ml with deionized water. The solution was stored in freezer until the test was performed. AAS was performed in Perkin Elmer absorption spectrometer to quantify the calcium concentration in the solution. The equipment was calibrated with four different standards containing 1.0, 1.5, 2.5 and 5 mg/L of  $\text{Ca}^{2+}$ . TRIS was used as blank. The slope and correlation coefficient were calculated for the calibration curve and are presented in table 5 below:

**Table 5:** Showing slope and Correlation Coefficient of blank

Test Sample	Slope	Correlation coefficient
Blank 1	0.07853	0.99688
Blank 2	0.07563	0.99482

### 3.9 Fourier transform infrared spectroscopy

Fourier transformed infrared spectroscopy (FTIR) was performed using Perkin Elmer Spectrum one FTIR spectrometer in Attenuated Total Reflectance (ATR) mode to determine the structural changes occurring in the composite upon immersion. The resolution

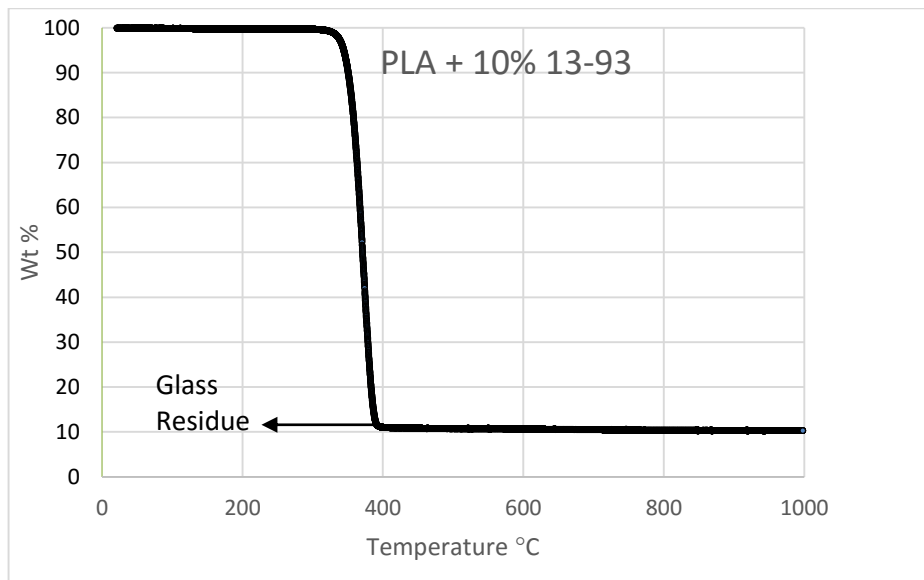


used was  $2\text{ cm}^{-1}$ , and the spectra was obtained from the accumulation of 4 scans. All spectra were baseline corrected and normalized to the band with maximum intensity.

## 4. RESULTS AND DISCUSSION

### 4.1 Thermogravimetric Analysis

Thermogravimetric Analysis (TGA) was performed post-processing and prior *in vitro* test in order to estimate the filler content in the test sample. Figure 6 shows the TGA curve for PLA+10% 13-93, taken as an example. As PLA is very sensitive to temperature, thermal degradation of polymer occurs during the TGA test. Thermal degradation of polymers starts from 320 °C and polymer degrades completely at 400°C, as seen by steep weight loss, thereby leaving the glass residue as shown in Figure 6.



**Figure 6:** Thermal degradation of composite

The glass residue represents the actual amount of glass particles in the composite sample. In Table 6 are compiled the actual filler content for all the tested samples. The obtained values are the average of two parallel samples. For composites containing both silicate and phosphate glass, the filler content was found to be close to the expected one and within the accuracy of the measurement. However, it was note-worthy to state that although feed rate of glass and polymer was same for both phosphate and silicate based composites as can be observed from Table 2, resulting composites have different amount of filler content. Equal amount of filler content was observed in both phosphate and silicate based composites that were loaded with 10 % bioactive glass. While the filler content for phosphate based composites was significantly lower for the samples with higher particles loading than silicate based composites. One of the possible reason could be that phosphate glasses typically have higher density than silicate glass. Introduction of Strontium oxide (SrO) into the glass causes an increase in density that correlates well with the

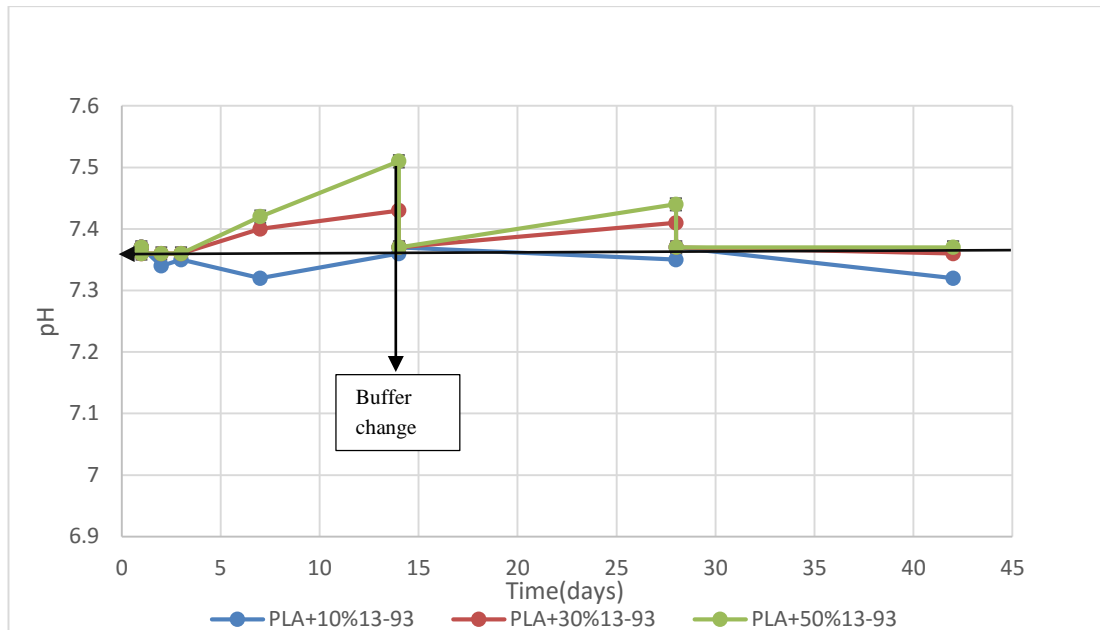
SrO concentration (Abou Neel *et al.* 2009) The higher density can lead to faster precipitation of the glass at the bottom of the extruder, resulting in lower amount of filler in the test sample than expected despite of same federate used during the extrusion process. Another, source of error could be the small sample size used in the TGA. Indeed, the sample tested was about 20 mg and the particles was 125-250  $\mu\text{m}$ . an alternative test that could be conducted is a burning test.

**Table 6:** Expected and experimental filler content in PLA (in wt. %). Two parallel samples were used for each polymer/bioactive glass composition.

Composites	Expected glass wt. %	Obtained glass wt. %	S.D.
PLA+ 10% 13-93	10	11	0.7
PLA+ 30% 13-93	30	33	1.4
PLA+ 50% 13-93	50	51	0.7
PLA+ 10% SR 50	10	12	0.7
PLA+ 25% SR 50	25	23	0.7
PLA+ 35% SR 50	35	34	1.4

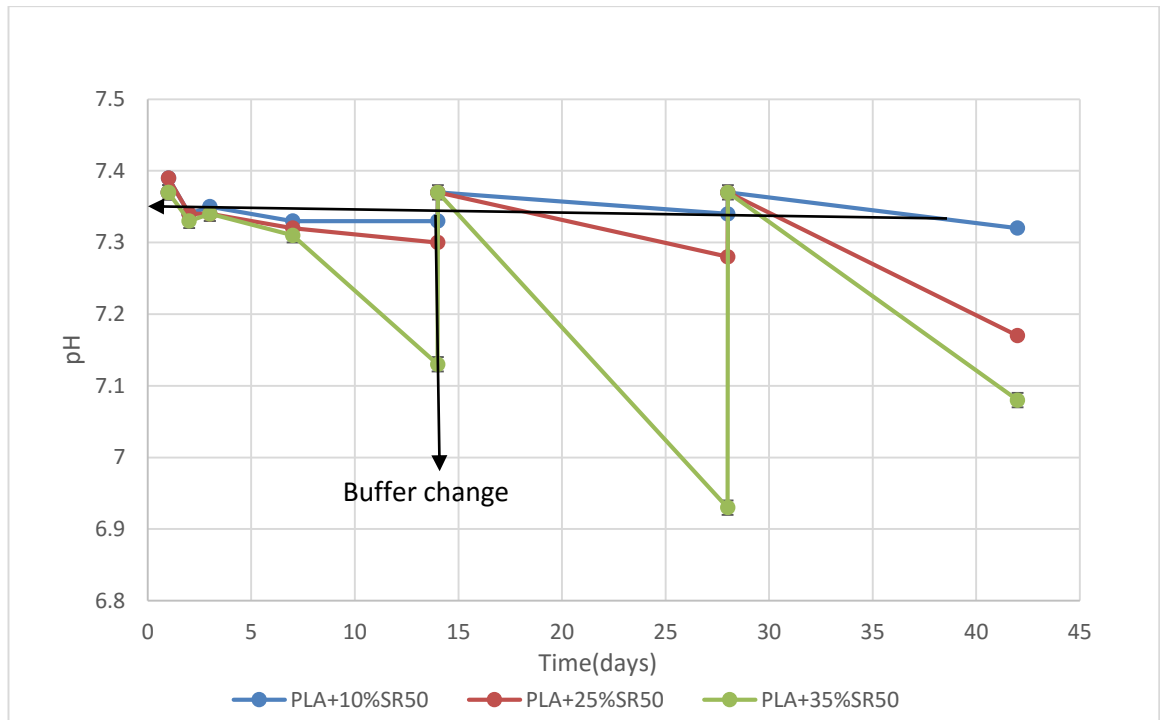
## 4.2 *In vitro* pH changes

Rods of 70 mm in length and 2.2 mm in diameter were immersed in TRIS buffer solution for 6 weeks. The solution was changed every two weeks and thus after changing the solution pH was 7.36 at 37 °C as indicated by horizontal line in Figure 7 and Figure 8. The pH of the buffer solution was recorded as a function of immersion time and is presented in Figure 7 for the PLA/silicate glass composite and Figure 8 for the PLA/phosphate glass composite.



**Figure 7:** pH changes of buffer due to silicate based composite. Each point represents the average of three parallel samples.

In the case of PLA/silicate glass composite, the pH slightly decreased with time, in the case of the composite filled with 10 wt. % of bioactive glass. However, with an increasing the glass content an increase in the pH was seen. This increase in pH is found to be higher after the first 2 weeks of immersion. The rise in pH was less at the 4 weeks and negligible at 6 weeks of immersion. Silicate bioactive glasses are known to react in physiological media by first exchanging  $H^+$  ions with  $Na^+$  ions and then  $Ca^{2+}$  is leached out. The release of alkali and alkaline earth ions lead to an increase in the pH. (Hench *et al.* 2004) The decrease in the pH seen for the 10 wt. % containing composite, may be due to the early degradation of the PLA. However, change in pH is typically reported to occur at later immersion time (Niemelä, 2010, Lu *et al.* 2002). Early degradation of the polymer may occur due to water accumulation at the glass/polymer interface. The lower pH change after the first 2 weeks of immersion may be due to either 1) decreased glass degradation over time or ii) contemporaneous glass (basic) and PLA (acidic) dissolution which in turns will lead to pH stabilization.



**Figure 8:** pH changes of buffer due to phosphate based composites. Each point represents the average of three parallel samples.

For phosphate glass based composites, pH was observed to decrease for all samples already at 24 hours. With an increase in the phosphate content the decrease in pH was greater, especially at the 2-week time point. Decrease in pH is expected in the case of phosphate bioactive glass dissolution. Indeed, upon dissolution, phosphate glasses leach out a large amount of phosphate which in aqueous solution forms phosphoric acid. Similar result has been observed by Georgiou, *et al.* 2006, while studying the *in vitro* test of PLA/phosphate glass composite foam. However, different dissolution media, for example deionized water was used in that case. The release of  $\text{PO}_4^{3-}$  was more prominent relative to the cations, indicating a rapid breakdown in the phosphate network of the glass. The release of  $\text{PO}_4^{3-}$  increased significantly with glass content.

Furthermore, Bunker *et al.* proposed that phosphate glasses dissolve in two stages. The first stage is controlled by the rate at which water diffuses into a volume of the bulk glass surface. This kinetics is obeyed only until the polymer chains at the surface are entirely surrounded by water. Totally hydrated chains can then disentangle from the partially hydrated chains still attached to the surface and float off into solution resulting in uniform glass dissolution and kinetics that are linear with time. Bunker *et al.* also mentioned that phosphate chain ends can be interconnected by hydrogen bonding which reduces the degradation rate (Bunker *et al.* 1984). The decrease in pH was found to occur at every two weeks' time points, after the buffer has been refreshed. It is noteworthy that typical phosphate glass does not generally induce such drastic decrease in pH (Massera, *et al.* 2012).

Thus it is reasonable to think that the accumulation of aqueous solution at the glass /PLA interface, which has a locally low pH due to the degradation of the glass further increased the PLA degradation which in turn leads to a more drastic pH drop. In contrary, no significant degradation of PLA was observed while doing *in vitro* test of  $\beta$ - TCP/PLA96 even upon up to 68 weeks, that would result in decrease in pH from the acidic degradation of PLA as observed in this study. (Niemelä *et al.* 2005). Similar result was obtained by Ahola *et al.* (2013). Hence, it should be noted that degradation behavior of polymer is highly dependent upon the types of buffer that have been used in *in vitro* test. Most of the polymer/bioactive glass composites that have been studied so far was done in PBS buffer which showed different degradation effect in polymer than TRIS buffer that is used in this thesis. So, in-depth study of degradation of polymer/bioactive glass in different dissolution medium might help to know the degradation phenomenon of polymer.

### 4.3 Water Absorption

Upon immersion in the TRIS buffer solution, the samples were found to swell as seen in Figure 9 which shows a photograph of the PLA/phosphate glass composite prior and after immersion. This indicates that the composite is retaining the solution. The swelling of the samples was also found to be a function of the filler content.

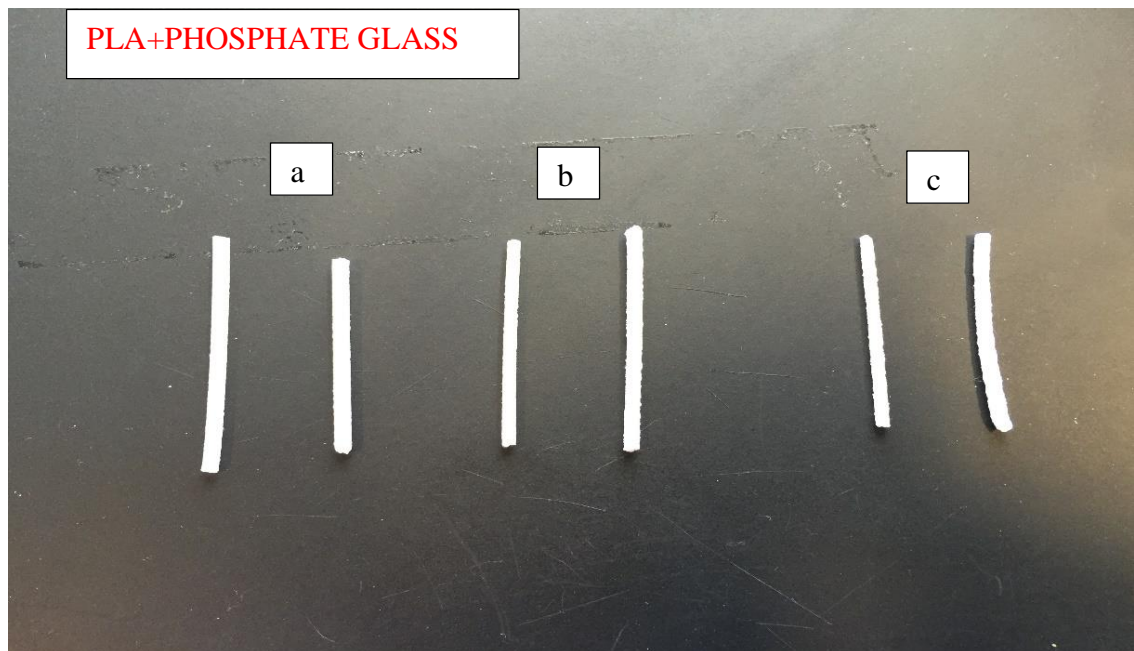
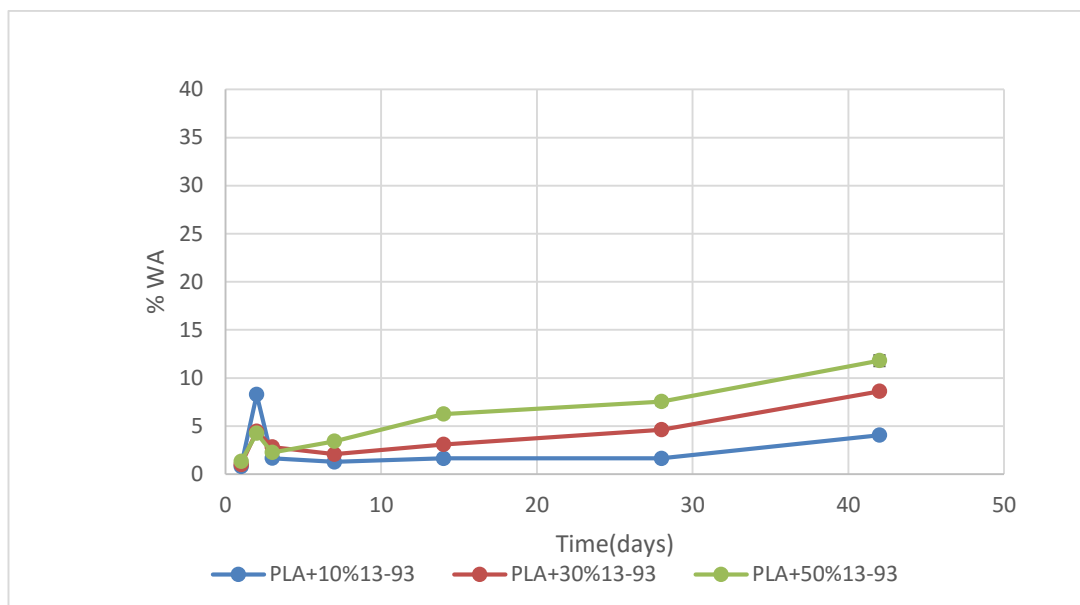


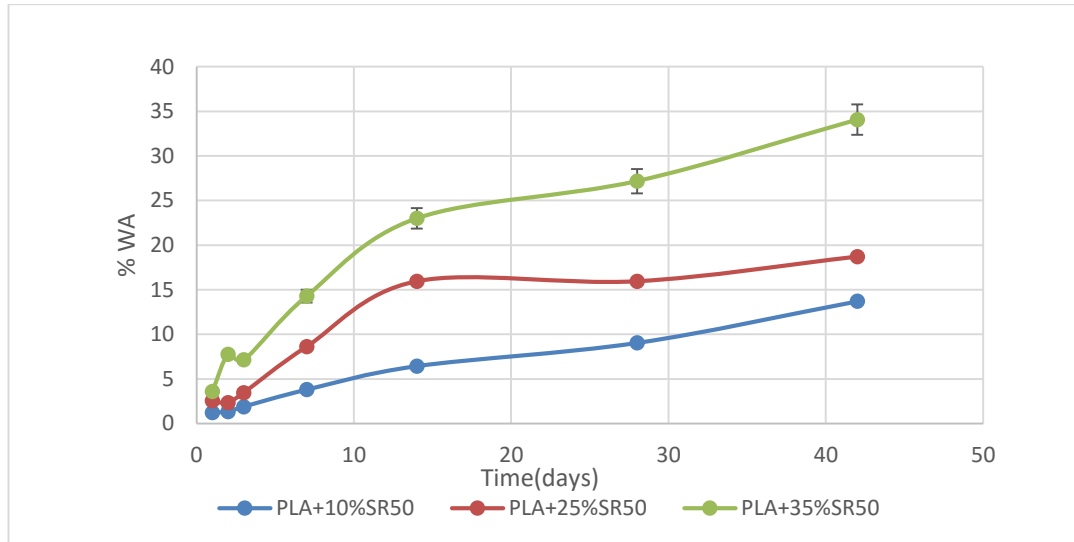
Figure 9: Visual Characterization of phosphate based composites. (a) PLA+10%SR50 (left 0wk right 6 wk) (b) PLA+25%SR50 (left 0 wk right 6 wk) (c) PLA+35%SR50 (left 0wk right 6 week)

The water absorption was further quantified using the equation (i) and (ii) presented in chapter 3. The Figure 10 below shows water absorption, in %, for silicate based composites



**Figure 10:** Water absorption by silicate based composites. Each points represents the average of three parallel samples.

From Figure 10, we can observe that WA increased for silicate based composite. Maximum water absorption was observed for PLA+10% 13-93 in 1 day which was surprising as for all other composites, WA was increased with filler content and upon increase in immersion time, this obtained result might be due to the measurement error for example improper calibration of weighing scale or composite samples were not wiped as carefully as it should be for these samples. However, accurate reason for the steep increase in WA at day 1 is not yet fully understood. WA was decreased at 3 days, after that WA was slowly increasing up to 6 weeks. Maximum water absorption was observed in PLA+50% 13-93 which may be due to the large amount of filler content in it. Indeed, an increase in filler content will lead to larger glass/polymer interface, site at which the water is more likely to be retained. Figure 11 shows the water absorption in phosphate based composite.



**Figure 11:** Water absorption by phosphate based composites. Each point represents the average of three parallel samples.

All sample started to uptake water as soon as they are immersed in the buffer for 1 day. The WA increased gradually with an increase in immersion time. The maximum uptake of water was seen in PLA+35% SR50 and, in general, the WA increased with increasing filler content. Hence, it was confirmed that WA increases with increase in filler content. This is because porosity of sample increases with increase in filler content where water gets more space to be absorbed.

Water absorption in silicate based composite was significantly lower in comparison to phosphate glass containing composite. This may be due to the large number of  $\text{OH}^-$  group at the silicate glass surface (Hence, 1991). The presence of  $\text{OH}^-$  group in glass are likely to increases the adhesion between polymer matrix and glass. The lower amount of  $\text{OH}^-$  group at the surface of phosphate glasses results in weaker mechanical interlocking between polymer matrix and glass. Such that interfaces between matrix polymer and filler particles enables more water to penetrate the structure resulting in increased water absorption.

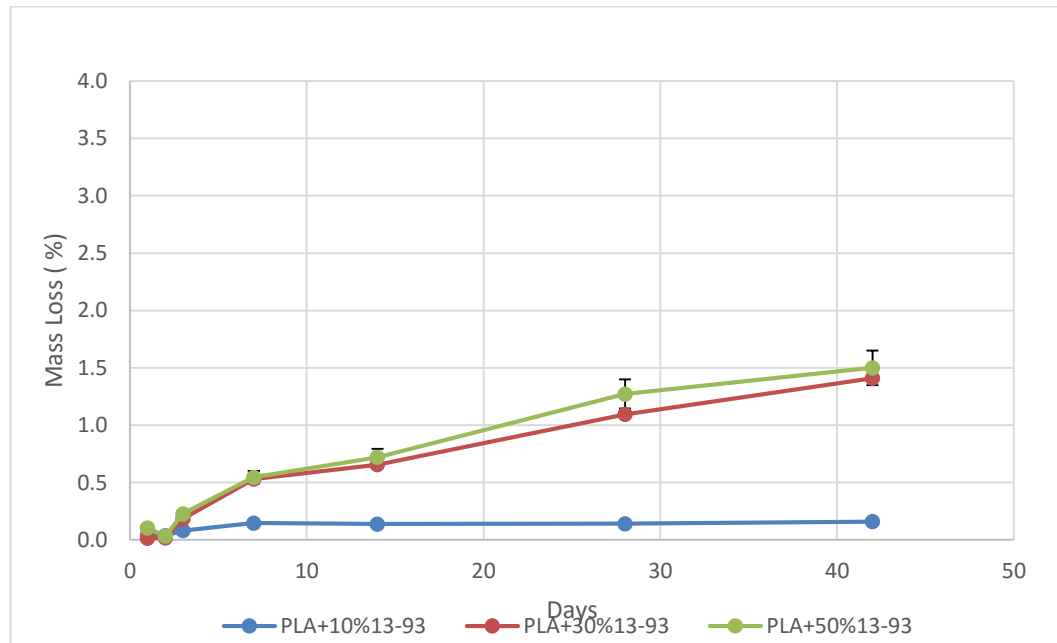
Niemelä, *et al.* (2010), also observed that the addition of osteoconductive filler increased the water absorption of the composites at the very early stage of hydrolysis in all sample studied which may be due to the presence of large number of porous structure that allowed more water to penetrate through the interface between polymer matrix and filler particles. Increase in both surface and interior porosity will be advantageous for bone tissue in-growth. (Kellomäki *et al.* 2000). It was reported that composites containing 13-93 bioactive glass absorbed more water than composite containing  $\beta$ -TCP which may be due to large number of pores that were formed on the surface of composite with 13-93 bioactive



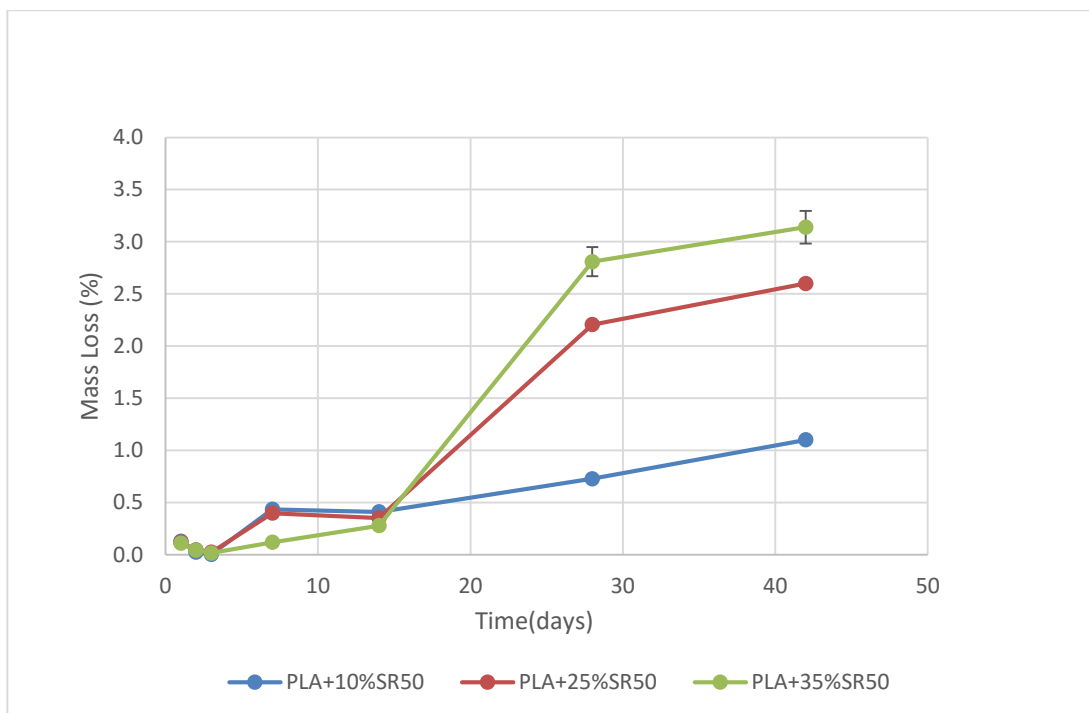
glass due to self-reinforcing process. (Niemelä, *et al.* 2010). Similar increase of water absorption was observed in other polymer/bioactive glass composites. (Niiranen *et.al* 2004, Maquet *et al.* 2004). Boccaccini and Maquet, (2003), also observed rapid increase in water up take by polymer/bioactive composites during first week of incubation. However, WA reached saturation level at 21 days and WA was gradually decreased.

#### 4.4 Mass Loss

The mass loss for silicate based composite is presented in Figure 12. Up to 72 h of immersion, no significant mass loss could be recorded. For longer immersion time the mass loss increases for the composites containing 30 and 50 wt. % of silicate glasses whereas is remained unchanged for the composite containing the lowest filler content. Figure 13 presents the mass loss of the composites containing phosphate bioactive glasses as a function of immersion time.



**Figure 12:** Mass Loss of silicate based composites sample. Each point represents the average of three parallel samples.



**Figure 13:** Mass loss of phosphate based composites. Each point represents the average of three parallel samples.

For Phosphate based composite, no significant mass loss was observed up to 2 weeks. After 2 weeks, there was a dramatic increase in mass loss with increase in immersion time. The mass loss was also found to increase with increasing the filler content. As can be observed from the Figure 13, maximum mass loss was observed at 4 weeks for composite containing 25% and 35% of glass and appear to level of for 6 weeks' immersion time.

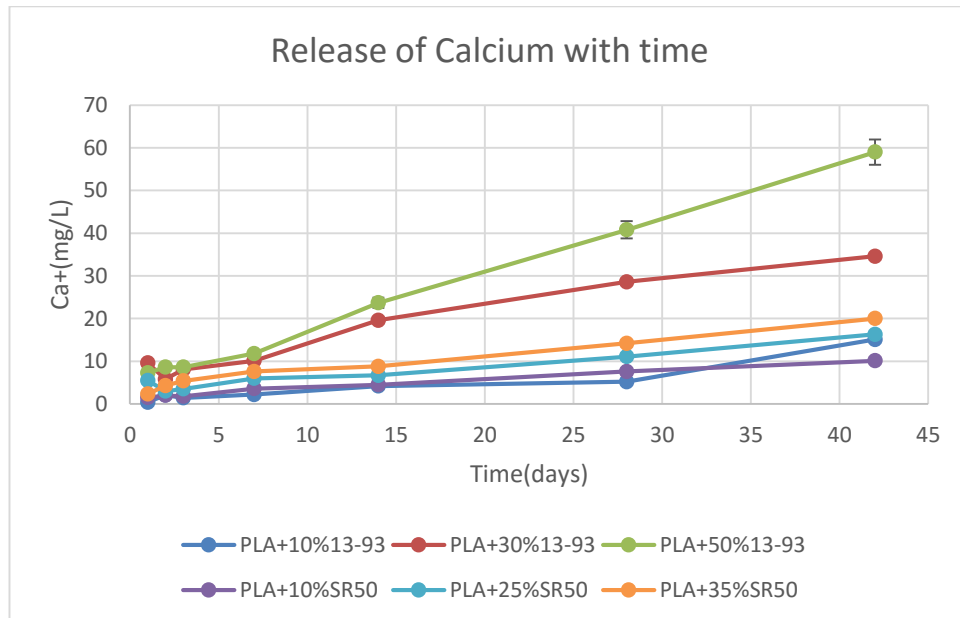
It was observed that mass loss in composites sample containing silicate glass was slower in comparison to sample containing phosphate glass. At the longest immersion time, the mass loss of phosphate containing PLA was at least two times higher than its silicate glass containing PLA counterpart. Mass loss in phosphate glass was higher due to weak interlocking between polymer and phosphate glass. The P–O–P bonds between the [PO<sub>4</sub>] units breaks under the attack of H<sup>+</sup> ions and water molecule, resulting in the destruction of glass network and disentanglement of short-chain polyphosphates into solution with different degree of polymerization. As the network breakage reaction of phosphate glass is highly sensitive to the attack of H<sup>+</sup> ions that usually decreases the pH value of aqueous media. Decrease in pH of aqueous media could certainly increase the dissolution rate of the phosphate glasses, resulting in rapid mass loss. (Gao *et al.* 2003)

The addition of  $\beta$ -TCP and 13-93 bioactive to self-reinforced samples reduced the overall degradation of composites in term of mass loss. Mass loss was observed at very beginning of immersion test for 13-93 bioactive glass based composite which may be due to rapid

dissolution of silicate glass while for  $\beta$ -TCP based composites, mass loss was observed at later stage. However, significant mass loss was started to observe after 52 weeks. (Niemi, *et al.* 2010). The mass loss was more pronounced until 48 weeks. The greater WA at the early stage of immersion probably induced the degradation of self-reinforced composites in terms of mass loss. (Niiranen *et al.* 2004)

## 4.5 Atomic Absorption Spectroscopy

The  $\text{Ca}^{+}$  release, which is an indication of the glass dissolution, was quantified by AAS. Figure 14 presents the cumulative dissolution of calcium ion up to 6 weeks for both silicate and phosphate bioactive glass containing PLA rods. The release of  $\text{Ca}^{+}$ , was increased with increasing immersion time and filler content in both case.  $\text{Ca}^{+}$  release was found to be higher for silicate glass based composite than phosphate glass based composite. This is because the dissolution of bioactive silicate glasses is primarily governed by the leaching of alkaline and alkaline earth ions within the solution while the Si is leached at lower rate forming a Si-rich gel. In the case of phosphate bioactive glasses, all ions in the glass network leach out at a similar rate and the surface of the glass does not change significantly its composition. As no gel layer is formed, the dissolution of phosphate based glass is more sustained. (Massera *et al.* 2012) Furthermore, it is likely that the dissolution rate of the silicate glass is higher than in the case of the phosphate one. Another eventuality for the lower Ca release in solution from the phosphate glass containing composite could be that, due to the higher P release the Ca is more likely to bind to P ions to form and precipitate a CaP layer.

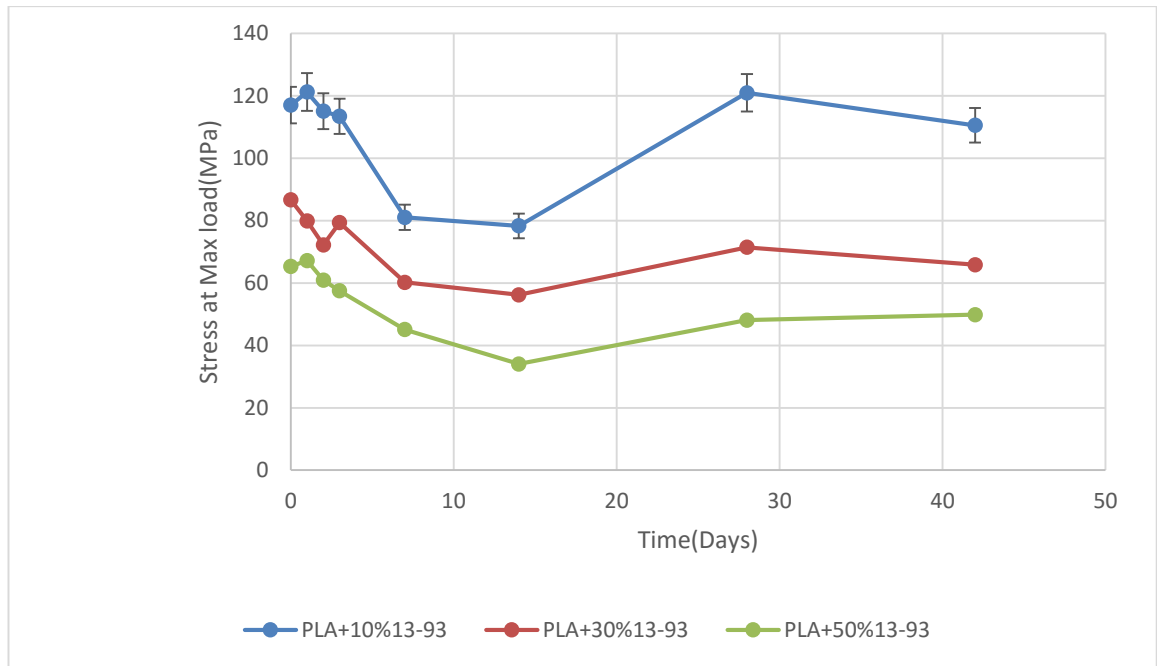


**Figure 14:** Calcium release profile. Each point represents the average of at least two parallel samples.

## 4.6 Mechanical Testing

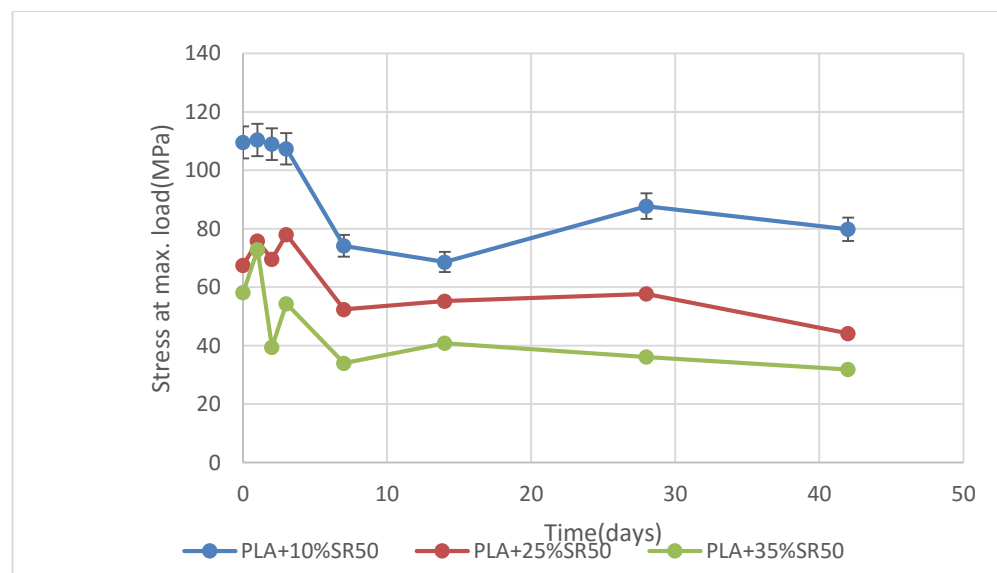
### 4.6.1 Bending Test (3 Point bending test)

The 3-point bending test was done for all 0 week as well as *in vitro* test samples. All the 0-week test sample were found to be brittle which was attributed by the brittle nature of glass. The brittleness of sample was found to be increased with increase in filler content. Similar result was obtained for PLA/bioactive glass composites. Sample became weaker and brittle with the increase of filler material. In order to improve initial mechanical property of sample and to decrease the brittleness of the composites self-reinforcing was done. (Niemelä, 2005). Silicate containing composites sample have highest initial bending strength (116 MPa) which can be observed in Figure 15. The bending strength decreased with *in vitro* test up to 2 weeks which is due to the degradation of composite especially degradation of glass for all *in vitro* test samples. Fastest decrease in bending strength was observed for PLA+50% 13-93. At the same time, test sample started to become ductile from brittle which is probably due to formation of HA layer on the surface of test samples. At 4-week test point, the bending strength increased drastically which was not expected. The result of bending strength was surprising at 4-week time point. The increase in bending strength may be due to the formation of HA. Then slight decrease in bending strength was observed at 6-week test point. Longer immersion test is required to be conducted to studying the mechanical behavior of composite in terms of bending strength. (Niemelä, 2008) reported result for bending strength of for similar polymer/glass composites which was different than here. Bending strength of composites was gradually decreasing from 117 MPa (0 wk) to 112 MPa till 6 weeks unlike in this study. The dramatic decrease of bending strength was observed only after 30 weeks of immersion test. Bending strength of composites was decreased upon increase in immersion time. Also, higher amount of filler content resulted in faster loss of mechanical properties of sample. The composites lost all of its mechanical strength at 65 weeks. (Niemelä, 2008)



**Figure 15:** Bending Strength of Silicate based composites. Each test points represents the average of three parallel samples.

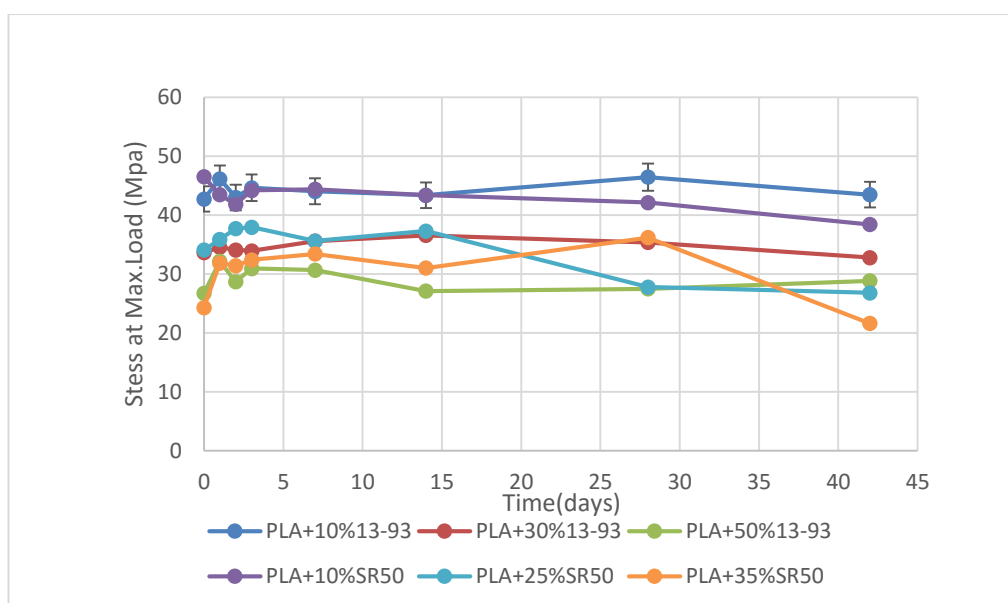
Phosphate based composites also behaved in the similar manner. However, the increase in bending strength at 4 weeks is not as significant as in silicate based composites. This may be due the reason that HA precipitation in phosphate glass may occur in later stage.



**Figure 16:** Bending Strength of Phosphate based composites. Each point represents the average of three parallel samples.

### 4.6.2 Shear Test

Shear strength did not change significantly over the time for both silicate and phosphate based composites. Shear strength was expected to decrease with increase *in vitro* test. Slight decrease in shear strength was probably due to water uptake and / or due to loosening glass particles from the sample. Shear stress was likely to decrease upon increase in *in vitro* test point. (Niemelä, 2005) Longer *in vitro* test are required to observe the behavior of shear strength over the time. Also, Niiranen, *et al.* (2004), observed decrease in shear strength depends upon immersion time. Further, shear strength was dependent upon filler content in the composites. Increase in filler content decrease the shear strength of the test samples.

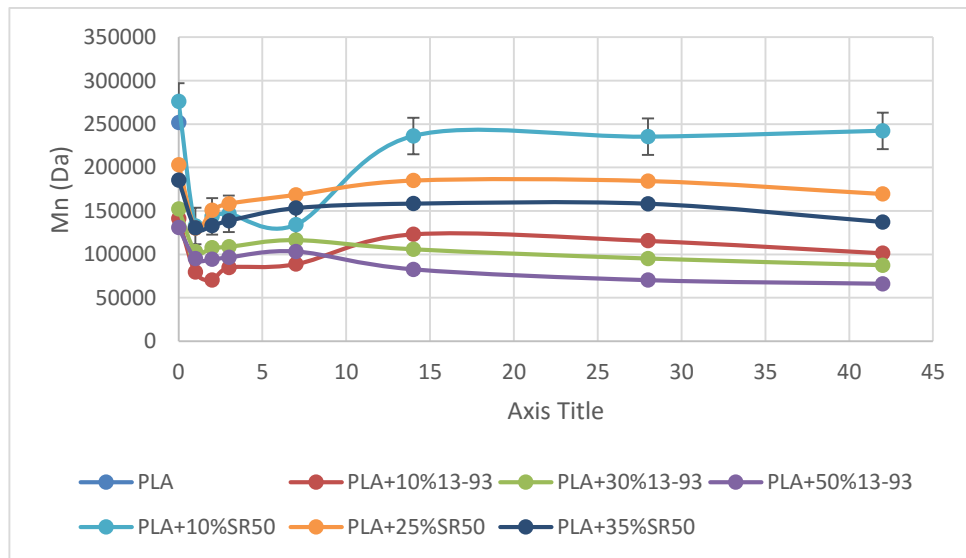


**Figure 17:** Shear Strength of Silicate and Phosphate based composites. Each point represents the average of three parallel samples.

### 4.7 Gel Permeation Chromatography

Molecular weight plays an important role in degradation of the composites studied. Figure 18 shows the number average molecular weight ( $M_n$ ) of polymer. The molecular weight distribution of all the test samples were monomodal throughout the whole hydrolysis. From Figure 18 below, it can be observed that the molecular weight of polymer decreases as soon as it is immersed in buffer. The decrease in molecular weight was started to observe at 24 hour of immersion time for all test sample. This is due to water diffusing into the polymer, especially in polymers amorphous structure. The penetration of water breaks the long polymer chain in shorter one resulting in decrease in molecular weight. (Niemelä, 2005) The molecular weight of silicate based composite decreased up to 2 weeks and became almost consistent up to 6 weeks. This may be due to degradation of polymer has

become slower. However, for phosphate based composites the decrease in molecular weight were drastic in comparison to silicate based composites. After that, the molecular weight of polymer gradually increased up to two weeks. There was a dramatic increase in molecular weight in context of phosphate based composite than in silicate based sample which can be clearly observed from Figure 18 below. The maximum increase of molecular weight was observed in PLA+ 10% SR50. The results obtained was unexpected as increase in molecular weight have not been observed yet for any of the polymer/bioactive glass that have been studied so far. This indicates that Phosphor content on glass has great effect on polymer chain which is needs in-deep analysis to interpret the result.

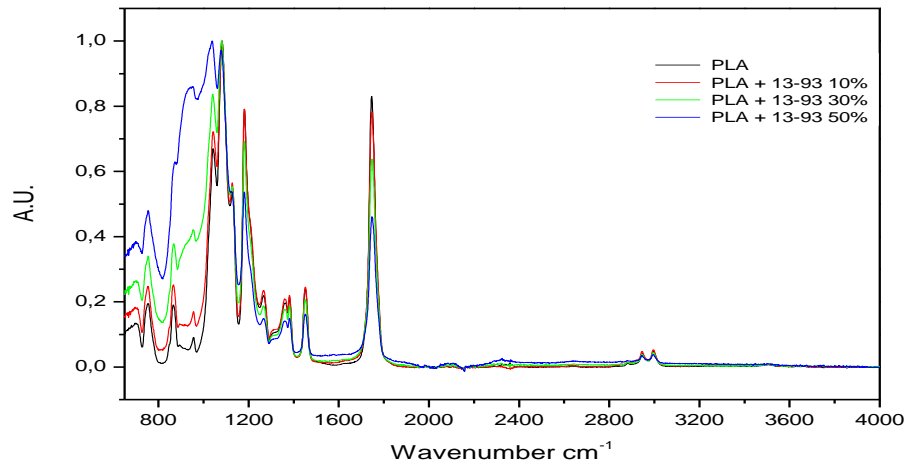


**Figure 18:** Number average molecular weight( $M_n$ ) of polymer/bioactive glass composite. Each point represents the average of two parallel samples.

## 4.8 FTIR

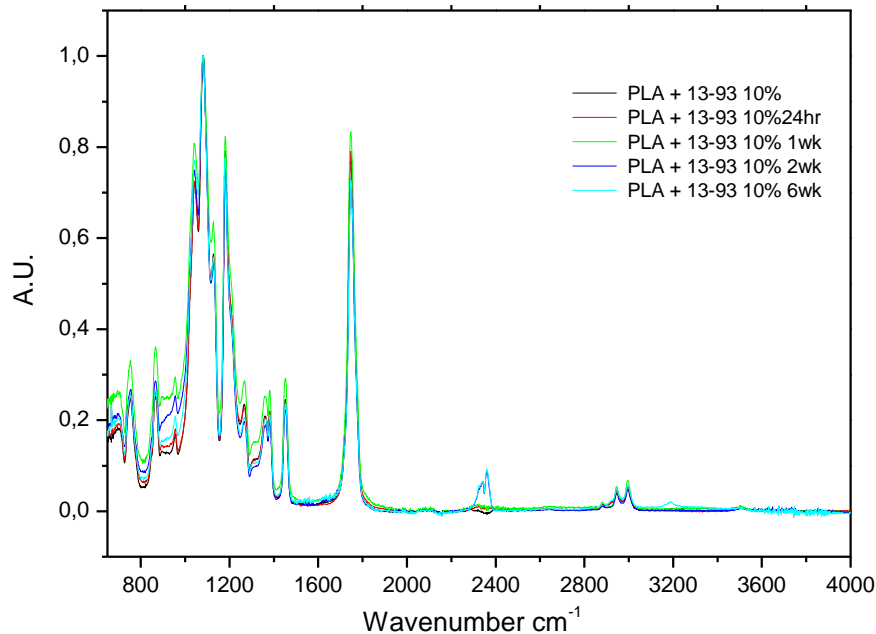
### a) FTIR spectrum of silicate based composite

FTIR spectrum of PLA is shown in Figure 19. The spectrum presents a strong absorption band at  $1748\text{ cm}^{-1}$  which is attributed to the stretching vibrations of amorphous carbonyl groups (Furukawa *et al.* 2007). The other bands that are observed at  $1452\text{ cm}^{-1}$  and  $1382\text{ cm}^{-1}$ , are due to the  $\text{CH}_3$  asymmetric and symmetric deformations. The strong absorption band located at  $1180\text{ cm}^{-1}$  is due to C-O-C stretching mode, while a shoulder positioned at  $1210\text{ cm}^{-1}$  is attributed to C-O-C stretching characteristic for the crystalline phase of PLA. (Buzarovska & Grozdanov, 2010)



**Figure 19:** FTIR spectrum of PLA + silicate glass composites(0 week)

Upon addition of silicate glass, 13-93, the spectrum showed resonances at 1050 and 940  $\text{cm}^{-1}$  attributed to the stretching vibration modes of Si bonded to non-bridging O in the glass network (Fu, *et al.* 2007). With increasing in the filler content, the band that are attributed to stretching vibration of the silica networks broadened. The decrease in peak intensity at band 1748  $\text{cm}^{-1}$ , 1452  $\text{cm}^{-1}$  and 1382  $\text{cm}^{-1}$  was observed with increase in filler content which may be due to the Si replacing the carbonyl group of PLA.

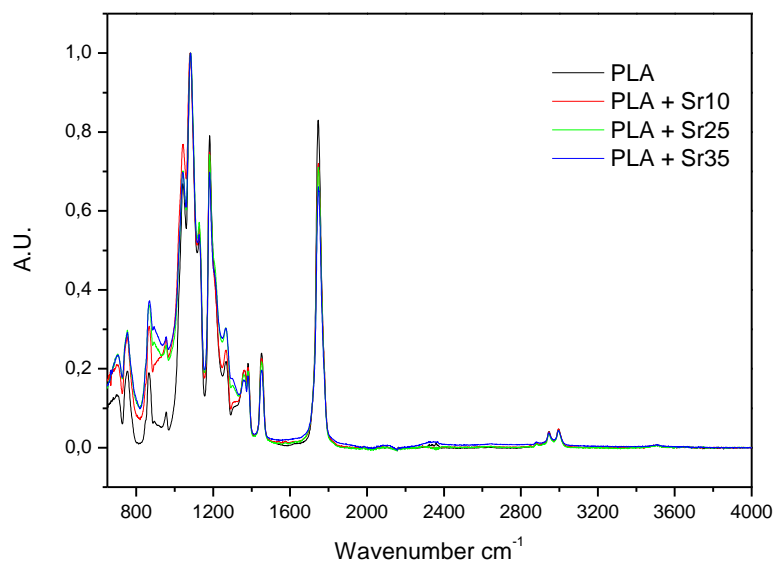


**Figure 20:** FTIR spectrum of in vitro silicate based composites

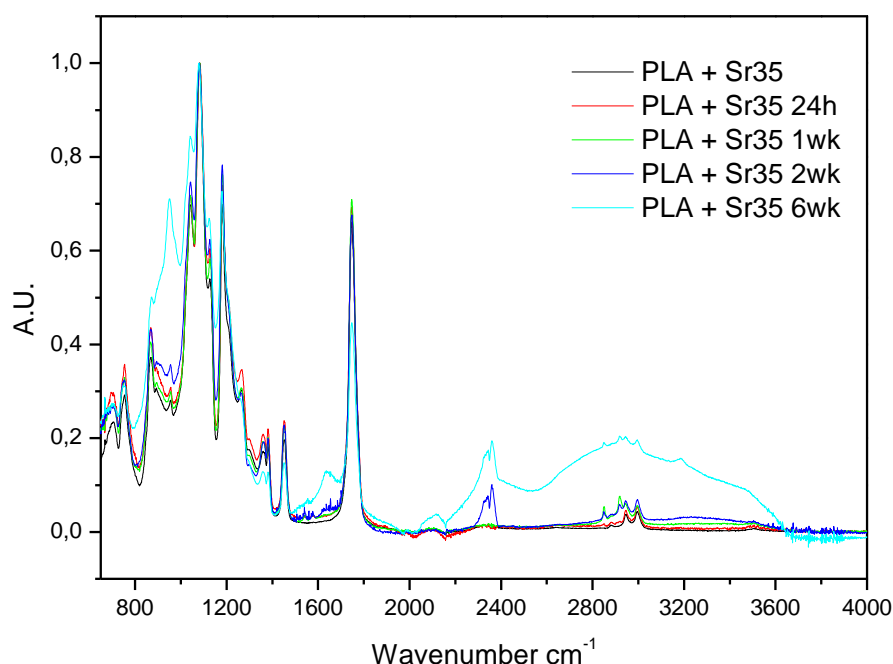


Figure 20 presents the FTIR spectra of the silicate based composites at various immersion time. Upon immersion of composite, no significant change was observed for PLA+ Silicate glass in this figure. The broadband centered at  $3400\text{ cm}^{-1}$  corresponded to O–H band. (Fu et al.2013) This absent of this band assures that there was no significant water absorption observed for PLA+10% 13-93 composites.

#### b) FTIR spectrum of Phosphate based composite



**Figure 21:** FTIR spectrum of PLA + phosphate glass composites(0 week)



**Figure 22:** FTIR spectrum of phosphate based composite *in vitro*

Figure 21 presents the FTIR spectra of the PLA-silicate glass composites. The typical FTIR of phosphate glass represents the band at 880  $\text{cm}^{-1}$  is attributed to P–O–P asymmetric stretching of bridging oxygen. The shoulder centered at 980  $\text{cm}^{-1}$  and the band peaking at 1,085  $\text{cm}^{-1}$  correspond to the symmetric and asymmetric stretching vibration of  $\text{PO}_3^{2-}$ . The band at 1,085  $\text{cm}^{-1}$  can be attributed to an overlap between  $\text{PO}_3$  and  $\text{PO}_2$ . The shoulder at 1,154  $\text{cm}^{-1}$  correspond to symmetric and the absorption band at 1,260  $\text{cm}^{-1}$  correspond to asymmetric vibration of  $\text{PO}_2^{2-}$ . (Massera et al. 2012) However, such bands cannot be observed significantly at Figure 21, which may be due to use of non-uniform sample. Best FTIR spectrum can be observed from powder sample or a thin and flat surface which was not the case in the sample that was used in this thesis.

Figure 22 presents PLA-phosphate glass composites. With an increase in immersion time, a band at  $\sim 2800 \text{ cm}^{-1}$  appears and broaden with immersion. This band that appears from 2800  $\text{cm}^{-1}$  to 3600  $\text{cm}^{-1}$  is due to water absorption within the composite structure. Navvaro *et al.* (2005), observed similar bands at 3000–3500  $\text{cm}^{-1}$  region which was due to the water uptake by PLA/glass composite.

In conclusion, from the FTIR spectra it appears that composite containing phosphate bioactive glass are more prone to absorb water during immersion. Whereas addition of silicate leads to greater changes in the structure of the polymer itself.

## 5. CONCLUSIONS

Hence, from above results, it can be concluded that phosphate based composites are likely to decrease the pH of the solution due to the formation of phosphoric acid while composite containing silicate glasses are likely to increase pH of solution due to leaching of cations in the solution making solution more alkaline. From mass change, water uptake measurement and FTIR test, it was clear that the water absorption was increased with increase in filler content. The changes were more drastic with the phosphate based composites compared to the silicate one. Calcium release was increased with immersion time and the silicate glasses released more  $\text{Ca}^{+}$  than the phosphate glass. The release of the  $\text{Ca}^{+}$  in the solution is a clear indication that the solution will be saturated overtime and this is likely to lead to the precipitation of a HA layer.

The mechanical properties of all investigated glasses were found to first decrease, as expected upon degradation of the composite. However, it was noteworthy that the bending strength increased after two weeks of immersion. Such result was unexpected, especially since the shear strength was not found to greatly change over the course of the test, and calls for more in-depth analysis of the structural changes occurring at the glass and polymer surface upon extended immersion time.

Finally, the molecular weight of the polymer was found to be decreased upon immersion. For silicate based composites, molecular weight decreased up to two weeks and remained stable for longer immersion time. In contrary, for the phosphate based composite, the molecular weight was found to increase drastically after two weeks of immersion, raising the question of the impact of P release on the cross linking of the polymer chains.

Hence, we can conclude that degradation of polymer/bioactive glass composite is highly dependent on types of matrix, filler (quantity and particles size) and dissolution solution that is used for *in vitro* test. However, optimum amount of filler content is dependent upon particular application. Most importantly, longer immersion test is required to fully understand the degradation behavior of both polymer and glass from composites as well as to observe formation of HA layer on the surface of composites.

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